Transkranielle Magnetstimulation (TMS) - Einflussfaktoren auf die kortikale Erregbarkeit und Retest- Reliabilität der TMS

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Erklärung

Ich versichere, dass ich meine Dissertation *„Transkranielle Magnetstimulation (TMS) - Einflussfaktoren auf die kortikale Erregbarkeit und Retest-Reliabilität der TMS“* selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

(Ort / Datum) Unterschrift

Meinem Vater gewidmet

Inhalt

1. Liste der themenrelevanten Veröffentlichungen der Verfasserin

Hermsen A, Eienbröker A, Haag A, Mylius V, Hamer HM, Menzler K, Karakas E & Rosenow F (2014). Perioperative Changes in Cortical Excitability, Mood and Quality of Life in Patients with Primary Hyperparathyroidism: a Pilot Study using Transcranial Magnetic Stimulation (TMS), *Euro J Endocrinol, 170*, 201-209, doi:10.1530/EJE-13-0552

Menzler K[*], **Hermsen A[*]**, Balkenhol K, Duddek C, Bugiel H, Bauer S, Schorge S, Reif PS, Klein KM, Haag A, Oertel WH, Hamer HM, Knake S, Trucks H, Sander T & Rosenow F for the EPICURE-Consortium (2014). A Common *SCN1A* Splice-Site Polymorphism Modifies the Effect of Carbamazepine on Cortical Excitability - a Pharmacogenetic TMS-Study, *Epilepsia, 55*, 362-369, doi: 10.1111/epi.12515

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Hermsen A, Haag A, Duddek C, Balkenhol K, Bugiel H, Mylius V, Menzler K & Rosenow F (eingereicht). Test-Retest Reliability of Paired Pulse Transcranial Magnetic Stimulation in Healthy Men and Women

2. Einleitung

Die vorliegende Arbeit befasst sich mit der Messung der kortikalen Erregbarkeit mittels transkranieller Magnetstimulation (TMS) und der Erfassung potentieller Einflussfaktoren. Hierzu führte die Verfasserin drei Studien durch und publizierte diese.

Die Modulation der kortikalen Erregbarkeit spielt bei verschiedenen neurologischen Erkrankungen, besonders aber bei Epilepsien, eine Rolle, da bei einer verminderten Erregbarkeitsschwelle vermehrt epilepsietypische Aktivität (z.B. elektroenzephalografische Veränderungen) und/oder ein Anfallsereignis auftritt (Cantello et al., 2000; Reis et al., 2007). Eine non-invasive Methode zur Messung der kortikalen Erregbarkeit beim Menschen ist die TMS. Bei der Bestimmung der kortikalen Erregbarkeit haben sowohl statische als auch sich verändernde Faktoren einen Einfluss auf die Messung. In der vorliegenden Arbeit wurde entsprechend der Einfluss von Kalziumveränderungen bei Patienten mit Hyperparathyroidismus vor und nach einer Parathyroidektomie (nicht statischer Faktor) und die Auswirkungen eines genetischen Polymorphismus eines Natriumkanals (SCN1A) (statischer Faktor) in Abhängigkeit von einer Medikamenteneinnahme untersucht. Ein weiterer Teil der Arbeit evaluiert die Retest-Reliabilität verschiedener, auch in den beiden anderen Studien verwendeten TMS-Parameter, in einem großen Kollektiv von Gesunden.

Im Folgenden wird zunächst das Konzept der kortikalen Erregbarkeit und deren Beeinflussbarkeit (z.B. durch Hormone) erörtert. Die in den Studien verwendeten TMS-Paradigmen, deren Auswertung und vermutete physiologische Grundlage werden beschrieben. Die Anwendung der TMS bei Patienten mit Epilepsie, als eine Gruppe mit pathologisch geänderter kortikaler Erregbarkeit, im Sinne eines Verlustes der Inhibition oder einer Übererregbarkeit, und deren Beeinflussung unter der Einnahme von Antikonvulsiva wird exemplarisch dargestellt. Anschließend folgt die Skizzierung der Studien, inklusive der Datengewinnung und der Resultate. Die Bedeutung für die Messung und Interpretation der kortikalen Erregbarkeit mittels TMS wird abschließend diskutiert.

3. Kortikale Erregbarkeit und Prinzipien der TMS

Die moderne transkranielle Magnetstimulation (TMS) wurde im Jahr 1985 von Barker eingeführt (Barker et al., 1985). Erstmals konnte in einer Selbstanwendung nachgewiesen werden, dass ein magnetisch induzierter Strompuls zu einer sichtbaren motorischen Antwort (Muskelkontraktion) und Bewegungsartefakten führte. Es folgte die Entwicklung von Prototypen. Die damals neue Methode war gegenüber der bis dato verfügbaren elektrischen Kortexstimulation weniger schmerzhaft und beinhaltete weniger Risiken, so dass der Einsatz rasch zunahm und heute zur Routinediagnostik in der Neurologie zählt.

Die TMS basiert auf einer elektromagnetischen Induktion, bei der, ausgelöst durch einen elektrischen Stromfluss in einer Spule, ein passageres magnetisches Feld von ca. 1-2 Tesla entsteht, welches wiederum zu einem elektrischen Impuls im Hirngewebe führt (Epstein, 2008). Dadurch kommt es zu einer Depolarisation kortikaler Neurone und bei entsprechend hoher Impulsstärke zur Generierung eines Aktionspotenzials (Davy, 2008). Über die Erregung kortikospinaler Neurone kommt es dann zu einer Aktivierung monosynaptischer spinaler Motoneurone, die wiederum zu einer motorischen Antwort des peripheren Muskels führen (Davy, 2008). Diese Kontraktion kann visuell und physiologisch mittels Messung durch Elektromyographie (EMG) objektiviert werden. Die kortikale Exzitabilität wird so über die Amplitude der motorischen Antwort messbar.

Für Studien zur kortikalen Erregbarkeit wird meist eine Schmetterlingsspule aus Kupferdraht (auch „Achter-Spule“, hier mit 7 cm Innen- und 9 cm Außendurchmesser) verwendet, um bei geringerer Stimulationsstärke eine fokale Stimulation zu erreichen (siehe Abbildung 1; (Weyh&Siebner, 2007)). Das induzierte Magnetfeld dringt einige Zentimeter in das Gewebe ein und nimmt graduell mit der Tiefe ab. Um das Handareal optimal fokal zu stimulieren, wird die Spule in einem 45 Grad Winkel zur Sagittalebene über der vermuteten Stelle geneigt und parallel zur Schädeloberfläche ausgerichtet. Durch einen von posterior nach anterior induzierten Stromfluss wird das weiterleitende kortiko-spinale System transsynaptisch am effektivsten aktiviert (Brasil-Neto et al., 1992).

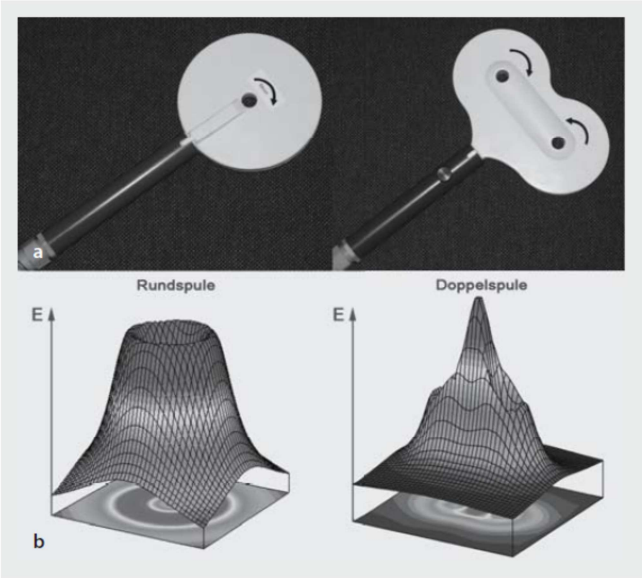

Abbildung 1. Rund- und Schmetterlingsspule und deren Verteilung des elektrischen Feldes (aus Weyh&Siebner, S. 24 in Siebner&Ziemann, Eds., Das TMS-Buch, 2007)

Der Proband bemerkt bei der TMS-Untersuchung ein Klickgeräusch bei Entladung der Spule sowie unter Umständen eine kurze Missempfindung an der Kopfhaut unter der Spule. Die TMS ist, vor allem in den für alle drei Studien verwendeten Doppel-Puls-Paradigmen, eine sichere Methode (Pascual-Leone et al., 1993). In seltenen Einzelfällen kam es durch die Untersuchung mittels TMS bei gesunden Probanden zu epileptischen Anfällen. Frequenz und Reizintensität entsprachen dabei jedoch nicht den für diese Dissertation verwendeten niedrigfrequenteren und reizärmeren Stimuli (Wassermann et al., 1996; Rossi et al., 2009).

4. Verwendete TMS Paradigmen und deren Auswertung

Für die Durchführung der in der Dissertation verwendeten Paradigmen, angewendet auf alle im Abschnitt 9 beschriebenen Studien, wurde auf ein im hiesigen Labor festgelegtes Protokoll zurückgegriffen, welches im Folgenden erläutert werden soll.

4.1 Testreiz (TR) und Ruhemotorschwelle (RMT)

Zunächst wird der *motor hot spot* bestimmt, also die Stelle des primär motorischen Areals, an der die motorische Antwort im Zielmuskel der Hand, M. abductor digiti minimi, maximal und am zuverlässigsten auslösbar ist. Zur Sicherung der Re-Positionierung wird diese mit einem Marker auf der Kopfhaut markiert. Der daraus resultierende Testreiz (TR) wird bei einer mittleren Amplitude von 1,5 mV notiert, um eine Vergleichbarkeit zwischen den Probanden zu gewährleisten. Es folgt die Bestimmung der Ruhe-Motor-Schwelle (*resting motor threshold*, RMT) bei nicht vorinnerviertem Muskel. Hierfür wird mithilfe eines statistischen Programms (Maximum-Likelihood Threshold Hunting, MLTH, F. Awiszus, Magdeburg) diejenige Stimulationsstärke als RMT definiert, bei der in 50% der Fälle eine Amplitude von 50 µV erreicht wird. Die Verzerrung durch mögliche Willkürpotenziale bei geringer Stimulation wird so minimiert. Hierzu werden 10 Reize einzeln appliziert und jeweils die Reizstärke gemäß dem Computerprogramm angepasst.

Die RMT ist bei intraindividuellen Verlaufsuntersuchungen in Studien als besonders stabile Größe gemessen worden (Boroojerdi et al., 2000; Malcolm et al., 2006; Plowman-Prine et al., 2008; Cacchio et al., 2009; McGregor et al., 2012) und wird daher oft als primärer Zielparameter bei longitudinalen Studien verwendet. Bei der Entstehung der RMT haben membranverändernde Mechanismen einen Einfluss, die die Entstehung von Aktionspotenzialen beeinflussen (Ziemann et al., 1996). Insbesondere die Aktivität von Natrium- und Kalzium-Ionenkanälen an der Zellmembran liegen der RMT zugrunde, wie pharmakologische Studien beweisen (Ziemann et al., 1996; Boroojerdi et al., 2001; Ziemann, 2004).

4.2 Kortikale Innervationsstille (Cortical silent period, CSP)

Ein weiterer Parameter der Messung der kortikalen Erregbarkeit mittels TMS ist die kortikale Innervationsstille (*cortical silent period*, CSP). Die CSP ist ebenfalls ein Einzelpuls-paradigma. Hierzu werden bei mit ca. 30% der maximalen Kraft vorinnerviertem Muskel, unter audio-visueller Prüfung und entsprechender Rückmeldung an den Probanden, insgesamt 20 Impulse mit einer Stärke von 110% der RMT appliziert. Die TMS führt zu einem passageren Ausbleiben der EMG-Aktivität. Die Zeit bis zum Wiederauftreten der Muskelaktivität wird als CSP definiert (Tergau et al., 1999). Die CSP ist somit ein Parameter der eine kortikale neuronale Inhibition messbar macht. Mit steigender Stimulationsstärke nimmt die Länge der CSP zu. In den Handmuskeln werden CSP um 200 ms Dauer gemessen (Cantello et al., 1992).

Der erste, frühe Teil der CSP (bis etwa 50 ms) wird durch spinale inhibitorische Prozesse, der spätere Anteil (ab etwa 50 ms) einzig durch kortikale Inhibition moduliert (Hallett, 2000; Wolters et al., 2008). Es wird angenommen, dass die Vermittlung über GABA-erge Mechanismen geschieht und somit zu einer intrakortikalen inhibitorischen Wirkung führt (Ziemann et al., 1996; Tergau et al., 1999; Reid et al., 2002).

Die Auswertung der CSP erfolgt klassischerweise offline. Für eine manuelle Auswertung wird die Zeit zwischen dem durch den TMS-Impuls entstehenden Stimulus-Artefakt und dem Wiederauftreten einer EMG-Aktivität von ca. 30% derjenigen EMG-Amplituden vor Stimulation gemessen. Zur Auswertung wurde das Programm Magnetix® genutzt. Die Dauer des CSP umfasst hier bereits die Stimulation (siehe Abbildung 2).

Abbildung 2. Screenshot der Auswertung der Dauer der CSP mittels Magnetix® (schwarzer Marker Beginn der CSP, pinker Marker Ende der CSP, Dauer hier insgesamt 179,6 ms). Die Marker werden per Hand gesetzt (visuelle Auswertung).

Für die Studie zur Retest- Reliabilität stand zusätzlich eine automatisierte Auswertung zur Verfügung, die auf der Methode von Garvey et al. (2001) basiert und für das Retest-Projekt programmiert wurde (CSPduration©, C. Bauer, Schopp). Hierbei werden die 20 individuell ermittelten Kurven per Durchgang rektifiziert und gemittelt. Gemäß dem Algorithmus von Garvey et al. (2001) wird die Dauer der CSP pro Proband, unter Berechnung des mittleren prä-Stimulus EMG, der mittleren konsekutiven Differenz und einer Konstanten von 2,66 ermittelt. Das Programm entfernt CSP-Werte, die kürzer sind als 30 ms oder erst 250 ms nach dem motorisch evoziertem Potenzial (MEP) beginnen. Der Anfang der CSP ist nach dem initialen MEP definiert und ist somit kürzer als die manuell ausgewertete CSP (s. Abbildung 3).

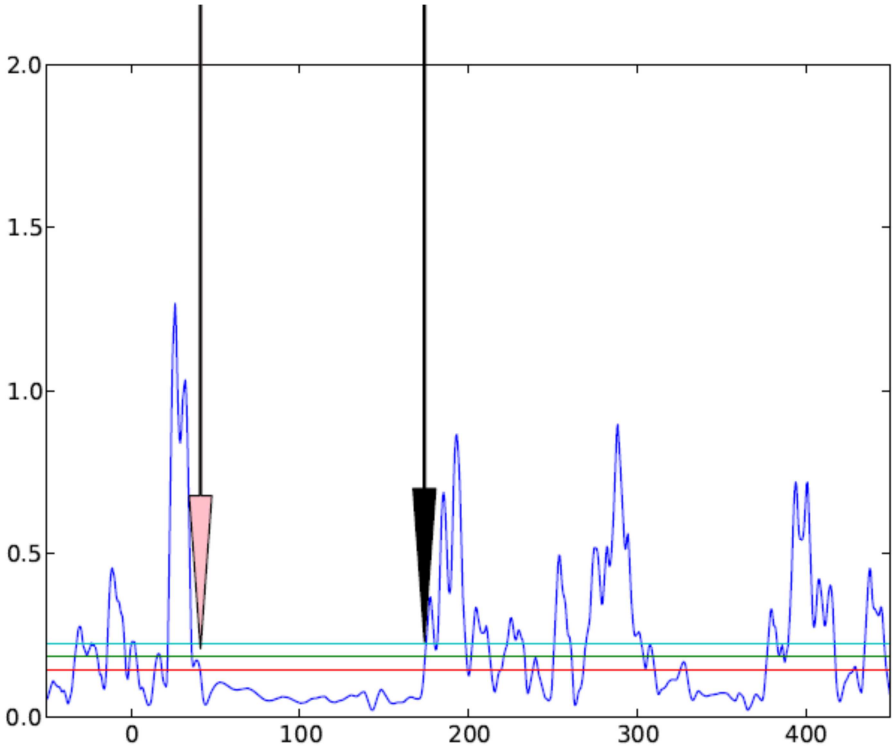

Abbildung 3. Darstellung der automatisierten Messung der CSP mittels CSPduration© (roter Pfeil Beginn der CSP, schwarzer Pfeil Ende der CSP, Dauer hier insgesamt 114,5ms).

4.3 Doppelpulsparadigmen: SICI, ICF und LICI

Des Weiteren wurden zwei verschiedene Doppelpulsparadigmen angewandt, die die kortikale Erregbarkeit mittels konditionierendem Stimulus kurzfristig ändern (Kujirai et al., 1993). Einem unterschwelligen ersten Reiz folgt ein überschwelliger zweiter Reiz bei entspanntem Zielmuskel. Die Zeit zwischen den Stimuli, das Interstimulusintervall (ISI) wird dabei variiert. Je nach Länge des ISI kommt es zu einer Hemmung oder einer Bahnung des durch den zweiten überschwelligen Teststimulus ausgelösten nachfolgenden MEP. Dieses ist in einer Amplitudenänderung des MEP sicht- und messbar. Der unterschwellige konditionierende Stimulus wird mit 75% der RMT appliziert, da bei dieser keine Beeinflussung der spinalen Rückenmarksneurone erfolgt (Kujirai et al., 1993; Di Lazzaro et al., 1998). Während kurze ISI von 3 bis 5 ms zu einer Hemmung führen (*short intracortical inhibition*, SICI), führen längere Intervalle (10 bis 15 ms) zu einer Bahnung (*intracortical facilitation*, ICF) (Brasil-Neto et al., 1992; Kujirai et al., 1993; Ziemann et al., 1996; Nakamura

et al., 1997; Werhahn et al., 1999). Noch längere ISI, zum Beispiel 150ms, führen wiederum zu einer Hemmung (*late intracortical inhibition*, LICI) (Nakamura et al., 1997). Für die hier durchgeführten Studien wurden je 15 Stimuli für die SICI (ISI von 3 bis 5ms) und für die ICF (ISI von 10 bis 15ms) und in einer der Studien ISI 150ms für die LICI gewählt. Diese wurden in randomisierter Folge durch das Computerprogramm generiert. Der Mittelwert der Amplitude der 15 Stimuli pro ISI wurde offline ausgewertet. Es folgt dann die prozentuale Wiedergabe:

$$ICF = \left[\frac{\text{Konditioniertes MEP (ISI 0ms)}}{\text{Unkonditioniertes MEP (ISI 10ms)}} \right] \times 100$$

Hierbei wird für die SICI dieser Wert von 100% abgezogen, so dass ein Wert von 100% eine vollständige Hemmung und ein Wert nahe 0% keinen Effekt des konditionierenden Reizes, sowie Werte unter 0% (negative Werte) eine Bahnung bedeuten.

$$SICI = 100 - \left[\frac{\text{Konditioniertes MEP (ISI 0ms)}}{\text{Unkonditioniertes MEP (ISI 10ms)}} \right] \times 100$$

Die SICI und ICF werden durch verschiedene kortikale Interneurone hervorgerufen und sind voneinander unabhängig (Hallett, 2000). Inhibitorische Signale (SICI) werden dabei am ehesten von GABA-ergen, exzitatorische (ICF) von glutamatergen Neuronen im Motorcortex vermittelt. (Kujirai et al., 1993; Ziemann et al., 1996; Ziemann, 2004). Die LICI inhibiert die synaptischen Überleitungen kortikospinaler Neurone (Nakamura et al., 1997).

5. Nicht-medikamentöse Einflussfaktoren auf die kortikale Erregbarkeit

Die kortikale Erregbarkeit ist durch verschiedene Größen beeinflussbar, von denen einige bereits gut untersucht wurden. Die oben genannte Anspannung von Muskeln (Di Lazzaro et al., 1998; Dominici et al., 2005), aber auch das professionelle Training der Muskeln, zum Beispiel bei Berufsmusikern (Rosenkranz et al., 2007), verändert die EMG-Antwort bei TMS. Bei einigen neurologischen Erkrankungen wurde darüber hinaus eine veränderte kortikale Erregbarkeit nachgewiesen, u.a. bei Schlaganfällen (Prashantha et al., 2013), Morbus Parkinson (Lefaucheur, 2005), und Epilepsie (s. Kapitel 6) - für ein Review zur Plastizität siehe (Cohen et al., 1998).

In der Literatur wird das Geschlecht aufgrund verschiedenartiger hormoneller Veränderungen, als Einflussfaktor auf die mittels TMS messbare Erregbarkeit diskutiert (Smith et al., 1999). So ist die Inhibition während der Zyklustage abhängig von ovulatorischen und anovulatorischen Zyklen (Hattemer et al., 2007). Fluktuationen in der Erregbarkeit wurden vor allem für anovulatorische Zyklen beschrieben. Andere Studien fanden hingegen keine Korrelation zwischen Geschlecht und TMS (Wassermann, 2002). Doch auch andere stoffwechselbedingte Veränderungen können die kortikale Erregbarkeit beeinflussen. Auf den Einfluss von Kalzium wird unter 7.1 eingegangen.

Einige Studien fanden einen Einfluss des Alters auf die gemessenen TMS-Parameter (Pitcher et al., 2003; Davidson&Tremblay, 2013). So war der TR zur Auslösung der MEP bei älteren Probanden höher als bei jüngeren und ebenso die RMT (Pitcher et al., 2003). Andere wiederum beschrieben keine Altersunterschiede in der Erregbarkeit, allerdings nur für männliche Studienteilnehmer (Smith et al., 2011). Eine weitere Studie fand ebenfalls keine Korrelation des Alters mit den TMS-Parametern (Wassermann, 2002). In diesem Zusammenhang wird auch die Beeinflussung durch eine veränderte Anatomie der Kalotte oder des Kortex diskutiert. Studien kommen dabei zu verschiedenen Ergebnissen; der individuelle Abstand Kalotte zu Kortex wird bei Gesunden zwar für nicht störend befunden (Danner et al., 2012), jedoch eine Tiefenkorrektur mittels metrischer Kalkulation (Stokes et

al., 2005) oder navigierter TMS empfohlen (Trillenberg et al., 2012). Ein möglicher Unterschied bei Stimulationszielen in der grauen oder weißen Hirnsubstanz wurde postuliert (Opitz et al., 2011). Die Eindringtiefe des elektrischen Feldes in die weiße Substanz sei größer als die der grauen Substanz.

Mögliche Einflussfaktoren der Messungen verschiedener Hemisphären und Interhemisphärenunterschiede (z.B. rechter vs. linker Motorkortex) wurden bei Gesunden meist nicht gefunden (Maeda et al., 2002; Cahn et al., 2003; Smith et al., 2011). Einige Autoren fanden jedoch eine niedrigere RMT (Macdonell et al., 1991; Triggs et al., 1994) oder eine verkürzte CSP (Macdonell et al., 1991) in der dominanten gegenüber der nicht-dominanten Hemisphäre. Triggs und Kollegen (1994) beschrieben dieses als physiologischen Nachweis der präferierten Händigkeit. Bei Erkrankungen die einer Hemisphäre zugeschrieben werden, z.B. Sprechstörungen, fanden sich sehr wohl entsprechende Erregbarkeitsunterschiede der Hemisphären (Alm et al., 2013).

Auch die Expertise und Fertigkeiten des Untersuchers wurden als relevanter Faktor diskutiert (Cacchio et al., 2009), sind bisher aber nicht systematisch untersucht worden.

6. Kortikale Exzitabilität, Epilepsie und Antikonvulsiva

In der Europäischen Union leiden ca. 3,4 Millionen Menschen an einer aktiven Epilepsie (Forsgren et al., 2005). Epilepsien sind damit eine der häufigsten chronischen neurologischen Erkrankungen. Ungefähr 1/3 der Betroffenen haben eine refraktäre Epilepsie und können nicht zufriedenstellend mit Antiepileptika eingestellt werden (Shorvon, 1996; Kwan&Brodie, 2000). Die genetischen Faktoren und deren Einfluss auf die Entstehung von Epilepsien sind relevant, aber noch nicht genügend erforscht (Schmidt&Loscher, 2005).

Bei einem epileptischen Anfall sind charakteristisch die abrupte, synchrone und repetitive Depolarisation größerer Neuronengruppen (Reis et al., 2007). Die zellulären Ursachen hierfür sind Gegenstand von Studien und bislang nicht eindeutig geklärt (Reis et al., 2007).

Die Epilepsien werden einerseits nach Ätiologie in idiopathische/genetische, symptomatische und mit unbekannter Ätiologie sowie andererseits nach Lage der epileptogenen Zone in generalisierte, fokale (lokalisationsbezogene) und nicht klassifizierbare Epilepsien unterteilt (ILAE, 1989), was auch für die Anwendung der TMS von Bedeutung ist. Während in therapeutischen Studien bei generalisierten Epilepsien in der Regel über dem Vertex stimuliert wird, wird bei fokalen Epilepsien durch Stimulation der vermuteten epileptogenen Zone und des homologen kontralateralen Areals ein Vergleich der Erregbarkeit der beiden Areale angestrebt. So kann die betroffene Hemisphäre mittels TMS detektiert und lateralisiert werden.

Im Folgenden werden lediglich die in dieser Dissertation verwendeten TMS-Parameter und mögliche Einflussfaktoren bei Epilepsiepatienten und verschiedenen Syndromen besprochen.

Bei idiopathisch/genetisch generalisierten Epilepsien (IGE) zeigt sich in der TMS-Messung eine niedrigere RMT in nicht behandelten Patienten, z.B. mit Juveniler Myoklonischer Epilepsie (JME), im Vergleich zu einer mit Valproat behandelten Gruppe (Reutens et al., 1993), so dass hierfür ein Medikamenteneffekt verantwortlich gemacht wird (Reis et al., 2007). Die JME scheint darüber hinaus über das Ausmaß der Änderungen der kortikalen

Erregbarkeit von anderen generalisierten Epilepsien mittels TMS-Messung unterscheidbar (Badawy et al., 2013). Ohne antikonvulsive Medikamente ist bei JME-Patienten im Vergleich mit anderen generalisierten Epilepsien die Ruhe-Motor-Schwelle erniedrigt und die kortikale Erregbarkeit in den ISI-Paradigmen erhöht. Insbesondere die JME weist eine Veränderung der transsynaptischen kortiko-spinalen Neurone im Sinne einer Übererregbarkeit auf, wie eine erniedrigte RMT beweist (Brigo et al., 2012). Bei Patienten mit JME zeigte sich nach Schlafentzug, welcher besonders bei diesem Syndrom Anfälle provoziert, die intrakortikale Bahnung (ICF) erhöht (Manganotti et al., 2006). Der Schlafentzug führte zudem zu einer Reduktion der RMT bei den JME-Patienten, während Gesunde keine Änderungen der kortikalen Erregbarkeit nach Schlafentzug zeigten (Manganotti et al., 2006).

Patienten mit progressiver myoklonischer Epilepsie zeigten bei unveränderter RMT und CSP, einen Verlust an späten inhibitorischen Prozessen (LICI) und eine frühe zugenommene Bahnung (Valzania et al., 1999). Aufgrund der normalen RMT und CSP sowie Hinweisen auf rhythmische Exzitabilität vermuteten die Autoren eine daher eine veränderte Erregbarkeit nur während der typischen myoklonischen Entäußerungen der Patienten.

Andere Epilepsiesyndrome, wie das schwer medikamentös behandelbare Lennox-Gastaut-Syndrom, gehen hingegen mit einer Abnahme der kortikalen Erregbarkeit, im Vergleich zu Gesunden und anderen Epilepsiepatienten, gemessen mittels TMS, einher (Badawy et al., 2012).

Bei fokalen Epilepsien wurde eine veränderte RMT als Effekt der antikonvulsiven Medikation interpretiert (Hamer et al., 2005). Bei Patienten mit fokalen Epilepsien, die nicht den motorischen Kortex betrafen, fand sich eine vom Fokus und Lateralisation abhängige Änderung der CSP. Die ipsiläsionelle CSP war verkürzt gegenüber der CSP der „gesunden“ Hemisphäre. Ebenso waren vor allem extratemporale Epilepsien von dieser Asymmetrie betroffen (Hamer et al., 2005).

Insgesamt scheinen Veränderungen in der intrakortikalen Hemmung bei Epilepsiepatienten mit fokalen Epilepsien zudem mit einer hohen Anfallsfrequenz und dem hochfrequentem Auftreten von epilepsietypischen Potenzialen im EEG zu korrelieren (Cantello et al., 2000).

Die TMS wird neben der Charakterisierung von speziellen Veränderungen in der Erregbarkeit bei unterschiedlichen Epilepsiesyndromen als wertvolles Instrument zur Darstellung physiologischer Medikamenteneffekte genutzt und Empfehlungen zur Anwendung der TMS für diesen Kontext existieren (Paulus et al., 2008). Eine umfassende Studie von Ziemann und Kollegen (2004) untersuchte den Einfluss verschiedener Antikonvulsiva (AED) auf die mittels TMS messbare kortikale Erregbarkeit (s. auch Abbildung 4).

Abbildung 4. Änderungen der kortikalen Erregbarkeit durch verschiedene Antikonvulsiva (AED).

AED		**Motorschwelle (MT)**	**Intrakortikale Erregbarkeit**	**CSP (Dauer)**
CBZ	Blockierung der spannungs-abhängigen Natriumkanäle	↑	=	↑
LTG	Blockierung der spannungs-abhängigen Natriumkanäle	↑	↓	=
PHT	Blockierung der spannungs-abhängigen Natriumkanäle	↑	n.d.	=
LEV	SV2A-Ligand und selektiver N-type-Kalziumkanalblocker	↑	=	=
TPM	Natriumkanalblocker, GABA-A-Rezeptor Agonist und non-N-methyl-D-aspartate (NMDA)-glutamate Rezeptor Antagonist, Carbo-anhydraseinhibition	=	↓	=
LCM	Blockierung der spannungs-abhängigen Natriumkanäle	↑	=	=

Tabelle modifiziert nach Ziemann (2004) und ergänzt. AED= Antiepileptic Drug (Antikonvulsiva), CBZ= Carbamazepin, LTG=Lamotrigin, LEV=Levetiracetam, TPM=Topiramat, LCM= Lacosamid, n.d.= nicht durchgeführt; ↑= Erhöhung bzw. Verlängerung; ↓= Reduktion;**=** = keine Veränderung durch AED

In Abbildung 4 findet sich eine nach Ziemann modifizierte Übersicht der Erregbarkeits-veränderungen durch AED. Es wurden mittlerweile auch die „neueren“ AED, wie Levetiracetam (Reis et al., 2004), Topiramat (Reis et al., 2002) und Lacosamid (Lang et al., 2013) untersucht und es wurden verschiedene Erregbarkeitsveränderungen nachgewiesen.

Während Levetiracetam die Erregbarkeitsschwelle (RMT) beeinflusste, wurde für Topiramat eine Beeinflussung der SICI gefunden. Lacosamid führt zu einer höheren RMT ohne eine Veränderung der CSP zu bewirken.

Für Carbamazepin (CBZ), ein Natriumkanalblocker, der in der Studie zum Gen-polymorphismus verwendet wurde, sind Veränderungen der Ruhemotorschwelle (RMT) und der Innervationsstille (CSP) bekannt (Ziemann, 2004). Die RMT steigt nach Einnahme von CBZ an, die Schwelle der Erregbarkeit nimmt also zu. Zudem bewirkt CBZ auch die Verlängerung der Dauer der CSP (Ziemann et al., 1996; Ziemann, 2004).

6.1 TMS als Therapie

Nur kurz soll der potenzielle Therapieeinsatz der TMS erwähnt werden, da hier keine Doppelpuls-Paradigmen, sondern die repetitive TMS (rTMS) verwendet wird. Neurologische Studien weisen auf positive Effekte in der Behandlung von Depression (George et al., 2013), und Schmerz (Lefaucheur et al., 2012; Mylius et al., 2012) hin. Die TMS wird zudem neuerdings auch als Instrument zur Vorhersage der Plastizität nach Schlaganfall eingesetzt (Torres et al., 2013).

Auch zur Behandlung von Epilepsiepatienten wurde die TMS bereits in randomisierten Studien verwendet (Fregni et al., 2006). Hierbei wird vornehmlich die niedrig-frequente rTMS eingesetzt (Theodore et al., 2002; Fregni et al., 2006; Cantello et al., 2007). Anfallsfreiheit wird meist nicht erzielt, so dass Erfolge der rTMS in Studien über die Anzahl der Responder (Anfallsreduktion um ≥ 50%) gewertet werden. Die Studien kommen insgesamt aufgrund ihrer Heterogenität in den Patientengruppen und Stimulationsparadigmen zu uneinheitlichen Ergebnissen. Am vielversprechendsten bezüglich einer Anfallsreduktion erscheint jedoch die fokale Stimulation neokortikaler Epilepsien (Tergau&Werhahn, 2007).

7. Studienskizzierung

Die nachfolgend beschriebenen Studien sind durch Mitarbeit der Verfasserin an allen Prozessen des wissenschaftlichen Arbeitens (Studienplanung, Durchführung, Datenauswertung, Schreiben der Publikation) entstanden. Thematisch handelt es sich um die transkranielle Magnetstimulation und deren Beeinflussbarkeit durch verschiedene Faktoren sowie die Retest-Reliabilität bei einem großen Studienkollektiv. Sämtliche Studien wurden durch die Ethikkommission des Fachbereichs Medizin der Philipps-Universität Marburg, bewilligt, beziehungsweise dem Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM, eudraCT Nummer der CBZ-Studie, s. 7.3; 2008-003392-40) vorgelegt.

7.1 Studie zur kortikalen Exzitabilität bei Patienten mit Hyperkalzämie

In der Studie von Hermsen und Kollegen (2014) wird anhand der Untersuchung von Patienten mit Hyperparathyroidismus (HPT) der Einfluss von Kalziumveränderungen auf die kortikale Erregbarkeit untersucht.

Kalzium (Ca^{2+}) hat aufgrund seiner Eigenschaften als intrazellulärer Transmitter einen direkten Einfluss auf das Neurotransmittersystem. Dabei spielt Kalzium auch eine Rolle in der Epileptogenese (Kulak et al., 2004; Raza et al., 2004; Thiel, 2006), weshalb spannungsabhängige Kalziumkanäle auch potenzielle Zielstrukturen von Antikonvulsiva sind (Stefani et al., 1997).

In einzelnen Fällen wurden epileptische Anfälle im Rahmen von pathologischen Kalziumveränderungen berichtet, die sowohl bei Hyper- als auch bei Hypokalzämie auftraten (Kanda et al., 1988; Kumpfel et al., 2000; Cherry et al., 2002; Castilla-Guerra et al., 2006). Bei primärem Hyperparthyroidismus (pHPT) liegt ein pathologisch veränderter Kalziumhaushalt durch eine Überfunktion der Nebenschilddrüse vor. Ursächlich hierfür sind meist Epithelkörperchenadenome. Die Diagnose wird dabei sowohl klinisch (u.a. Nierensteine, Knochenschmerzen, Depression) als auch laborchemisch gestellt. Nach einer Normalisierung des Ca2+ bei symptomatischen Patienten tritt häufig eine Besserung der Lebensqualität auf (Pasieka&Parsons, 1998; Coker et al., 2005; Mihai&Sadler, 2008;

Caron&Pasieka, 2009). Die einzige kurative Behandlung bei pHPT ist die Entfernung der einzelnen Adenome oder der hyperplastischen Nebenschilddrüse (PTx) (Hasse et al., 2002; Caron&Pasieka, 2009; Karakas et al., 2010). Hierdurch geht der Patient von einer Hyperkalzämie in eine Normokalzämie über.

Eine rezente Studie untersuchte akute und chronische Hyperkalzämie erstmals mittels rTMS und fand eine kurzfristig veränderte synaptische Plastizität (Iacovelli et al., 2011).

Letztlich ist daher die Rolle von Kalzium und die Wechselwirkung mit der kortikalen Erregbarkeit noch ungeklärt, da die Berichte sich vor allem auf klinische Fälle beziehen. Die Patienten mit PTx dienten somit als Model für eine rasche Änderung des Kalziumhaushalts von hyper- zu normokalzäm in dieser Pilot-Studie.

Für die vorliegende Studie wurden 20 Patienten rekrutiert. Aufgrund von TMS-Intoleranz (2 Patienten), fehlendem EMG-Signal (2 Patienten) und einer peripheren Nervenschädigung (1 Patient) reduzierte sich die Gruppe auf 15 Patienten (weiblich: 7; Alter 55,0 Median, Verteilung 22-73 Jahre). Diese wurden am Tag vor der Operation sowie im Median 3 Tage postoperativ erneut untersucht. Damit wurden potenziell überdauernde Effekte der Analgetika ausgeschlossen.

Gleichzeitig wurden Fragebögen zum subjektiven Empfinden bezüglich Depressivität (BDI-II, Beck Depressions Inventar (Beck et al., 1996)), zur Gesundheit (SF-36, Short Form Health Survey (SAGE Publications)) und zur krankheitsspezifischen Entwicklung (PAS, Parathyroid Assessment of Symptoms (Pasieka et al., 2002)) ausgefüllt. Diese wurden zudem 6 Monate post-OP per Post versandt. Der Kalzium- und Parathormonspiegel wurde prä- und post-operativ gemessen. Die Operation war in allen Fällen erfolgreich, was durch einen sinkenden Kalzium- und Parathormonspiegel bestätigt werden konnte (CA^{2+} prä: 2.8mmol (Verteilung: 2.6-2.9) vs. post: 2.3mmol (2.2-2.5)).

Betrachtet man alle 15 Patienten, fand sich in der Studie keine signifikante Veränderung der kortikalen Erregbarkeit von prä- nach postoperativ, gemessen mittels TMS, in keinem der

Parameter (Wilcoxon-Test, alle p>.1). Eine Beeinflussung der kortikalen Erregbarkeit durch einen Kalziumabfall ließ sich somit in unserem Kollektiv nicht nachweisen.

Der Kalziumspiegel korrelierte postoperativ negativ mit depressiven Symptomen (Spearman-Rang-Korrelation r= -.633, p=.027) und positiv mit einer Verbesserung krankheitsspezifischer Defizite (r=.809, p=.005), gemessen mit dem PAS (Parathyroid Assessment of Symptoms, (Pasieka et al., 2002)). Der Depressivitätsscore nahm bis zur 6- Monatsuntersuchung ab (prä: 9.6 ± 5.4; post: 7.6 ± 6.4, 6 Monate post-OP: 5.4 ± 4.8; p=0.05) und korrespondierend hierzu nahm die Lebensqualität in verschiedenen Parametern zu. Wir fanden hohe Korrelationen des unseres Wissens nach erstmals im deutschsprachigen Raum angewandten PAS mit dem weitverbreiteten Gesundheitsfragebogen SF-36 (SAGE Publications) zu allen drei Messzeitpunkten (s. Anhang, Nachdruck der Publikationen), welches die Anwendbarkeit des PAS weiter unterstreicht. Dieses sollte im Verlauf an einem größeren Patientenkollektiv für den deutschsprachigen Raum bestätigt werden. Die Fragebogendaten sollten des Weiteren in einer größeren Studie auch bei anderen chirurgischen Patienten evaluiert werden, damit eine genauere Eingrenzung möglicher unspezifischer Effekte einer OP gegenüber den Effekten einer Kalziumänderung erfolgen kann.

Ebenso ist eine Untersuchung von Patienten mit Hypokalzämie mittels TMS interessant, um die Auswirkungen eines niedrigen Kalziumhaushaltes auf die kortikale Erregbarkeit zu objektivieren.

7.2 Vergleichsstudie bei Gesunden mit zwei verschiedenen Genotypen eines SCN1A-Polymorphismus im Gen des Natriumkanals Nav 1.1 in Abhängigkeit von der Einnahme des Natriumkanalblockers Carbamazepin oder Plazebo

In der Studie von Menzler, Hermsen und Kollegen (2013) wurde ein möglicher genetischer Mechanismus einer Medikamentenresistenz an einem gesunden Kontrollkollektiv untersucht. Da bei 30-40% der Epilepsiepatienten keine ausreichende Anfallskontrolle durch medikamentöse Therapie erreicht werden kann, ist die Bedeutung genetischer Faktoren für die Pharmakoresistenz in den Vordergrund gerückt. Genetische Prädiktoren der Wirksamkeit

eines Antiepileptikums könnten darüber hinaus bei der Auswahl des geeigneten Medikamentes sowie bei der Dosisfindung nützlich sein.

Verschiedene Studien mit dem häufig bei Epilepsiepatienten eingesetzten Antiepileptikum (AED) CBZ fanden eine veränderte Tagesdosis in Abhängigkeit von einem Polymorphismus des *SCN1A*-Gens rs3812718 (Tate et al., 2005; Tate et al., 2006; Abe et al., 2008). Epilepsiepatienten mit dem Genotyp AA nahmen dabei die höchste CBZ-Dosis, Patienten mit GG die niedrigste (Tate et al., 2006; Abe et al., 2008). Dieser Zusammenhang zwischen den Genotypen und der mittleren CBZ-Dosis konnte in einer anderen Studie jedoch nicht bestätigt werden (Zimprich et al., 2008).

Carbamazepin ist in diesem Zusammenhang deswegen interessant, weil es zu einer Modifikation spannungsabhängiger Natriumkanäle führt. Es bindet an die Alpha-Untereinheit dieser Kanäle, die durch die Gene *SCN1A, -2A, -3A* und *-8A* kodiert werden (Tate et al., 2005). Der untersuchte Polymorphismus befindet sich auf dem *SCN1A*-Gen (IVS5N+5 G -> A; dbSNP: rs3812718), und liegt mit großer Häufigkeit in der Normalbevölkerung vor. Die Häufigkeit des homozygoten Genotyps AA beträgt 25%, des heterozygoten Genotyps AG 53% und des homozygoten Genotyps GG 22%.

Die Fragestellungen der Studie lauteten 1) ist der SNP rs3812718 assoziiert mit der Baseline Erregbarkeit der beiden Genotypen und 2) ergibt sich eine CBZ-abhängige Veränderung im Vergleich zu Placebo zwischen diesen. Eine Bestätigung ersterer Frage würde die pathophysiologische Bedeutung des *SCN1A* SNP rs3812718 in Bezug auf komplexe Epilepsien bekräftigen, während letztere dessen pharmakogenetische Relevanz unterstreichen würde.

In der durchgeführten Studie wurden gesunde Probanden genotypisiert (n=271) und die Probanden mit den homozygoten Varianten, als Extreme, wurden in den weiteren Studienverlauf eingeschlossen (n=140 (51%); AA=77 (28,4%), GG=63 (23.2%)). Ausschlusskriterien waren unter anderem zentralnervöse Medikation, neurologische (inkl. einmaliger Anfall) und psychiatrische Erkrankungen, Metallimplantate am Kopf, EKG-Auffälligkeiten und

Schwangerschaft. Es wurden nur Rechtshänder, ermittelt mittels Edinburgh Handedness Inventar (Oldfield, 1971) (EHI≥80), eingeschlossen. Aufgrund der CBZ-Gabe wurde zur Wahrung der Ausschlusskriterien bei allen Probanden vor der ersten Visite ein EKG und bei Frauen an beiden Visiten ein Urinschwangerschaftstest durchgeführt.

In einem doppelblinden, randomisierten und plazebokontrollierten Design wurden die nach drop-out verbliebenen Probanden (n=92, AA=49, GG=43) an zwei mindestens 14 Tage auseinanderliegenden Visiten je zweimal mittels TMS bezüglich ihrer kortikalen Erregbarkeit untersucht. Zunächst fand je eine Baselinemessung statt. Danach wurde randomisiert Placebo (PL) oder 400mg CBZ oral appliziert und nach 5 Stunden (Wirkmaximum von CBZ) wurde eine erneute TMS-Messung vorgenommen. Die subjektive Wirkung von CBZ wurde in einem anhand der bekannten Nebenwirkungen verfassten Nebenwirkungsscore standardisiert aufgezeichnet.

Die Probanden unterschieden sich nicht bezüglich Alter (AA: 23.5±2.7, GG: 24.2±3.9), Geschlecht (AA: 25 männlich, GG:27 männlich), Reihenfolge der Randomisierung (AA: PL-CBZ 27, GG: PL-CBZ 17) oder CBZ-Serumlevel (AA: 4.5±1.2, GG: 4.6±1.0, alle $p>.1$, t-Test für unverbundene Stichproben bzw. Chi-Quadrat-Test).

Wie erwartet fanden wir, unabhängig vom Genotyp, einen Einfluss von CBZ auf die kortikale Erregbarkeit (Ziemann, 2004). Die Stärke des TR nahm zu und die RMT stieg an, die CSP-Dauer nahm zu (respektive $p<.001$, $p=.002$, $p=.023$). CBZ zeigte somit die bekannten Effekte auf die kortikale Exzitabilität. Die Probanden berichteten 5 Stunden nach CBZ Einnahme mehr Nebenwirkungen als 5 Stunden nach PL Einnahme ($p<.001$). Diese beinhalteten vor allem leichte Schläfrigkeit, Koordinationsprobleme und Schwindel.

Wir fanden keine Baselineunterschiede in den TMS-Parametern zwischen den beiden Genotypen (alle $p>.1$).

Nach Einnahme von CBZ verhielt sich die kortikale Erregbarkeit der Probanden mit AA jedoch anders als die der Probanden mit GG (MANCOVA, multivariate Analyse $p=.029$).

Letztere zeigten, im Vergleich CBZ vs. Placebo unter Einbeziehung der jeweiligen Baselinemessung, eine stärker verlängerte CSP-Dauer, während die CSP-Dauer bei AA nach CBZ-Einnahme unverändert blieb (Univariate Analyse, p=.013).

Wir konnten damit eine funktionelle pharmakogenetische Beziehung zwischen den *SCN1A* SNP Genotypen und der Reaktion auf CBZ nachweisen. Die Effektivität von CBZ scheint somit genetischen Einflüssen zu unterliegen, welche wahrscheinlich auf einer Modifikation kortikaler GABA-erger Interneurone beruhen. Das Ausbleiben einer differenziellen Veränderung der RMT in unserer Studie sowie weitere Ergebnisse bei knock-out Mäusen für *SCN1A* legen, zusammen mit den neurophysiologischen Grundlagen der CSP, eine inhibitorische Wirkung der Interneurone am Natriumkanal $Na_v1.1$ nahe (Ogiwara et al., 2007; Martin et al., 2010).

Des Weiteren wurde die genotypunabhängige Veränderung der RMT und CSP als Bestätigung der in früheren Studien (Ziemann et al., 1996; Ziemann, 2004) beschriebenen Veränderung der spannungsabhängigen Natriumkanäle objektiviert.

Die Studie kann die zweite der oben beschriebenen Fragestellungen bezüglich der pharmakogenetischen Relevanz des *SCN1A* SNP rs3812718 positiv beantworten und legt als Grund für eine Pharmakoresistenz die sogenannte „Substrat-Hypothese“ nahe. Diese beinhaltet die verminderte Responsivität des *drug target,* zum Beispiel durch strukturelle Veränderungen, als ursächlich für ein schlechteres Ansprechen auf AED (Clancy&Kass, 2003; Ferraro&Buono, 2006). Ebenso wird die Wirkung von genetischen Faktoren im Zusammenhang mit Pharmakoresponsivität und deren Messbarkeit mittels TMS bestätigt.

7.3 Studie zur Retest- Reliabilität in einem großen Probandenkollektiv zu zwei Messzeitpunkten

In dieser Studie von Hermsen und Kollegen (eingereicht) wurde die Retest-Reliabilität verschiedener Einzel- und Doppelpulsparadigmen zu zwei Untersuchungszeitpunkten bestimmt.

Zur Retest- Reliabilität liegen bereits einige Studien vor, wobei jedoch meist nur wenige Parameter pro Studie untersucht wurden. Die RMT wurde häufig als reliabel befunden (Malcolm et al., 2006; Plowman-Prine et al., 2008; Cacchio et al., 2009; McGregor et al., 2012). Studien zur CSP lagen weniger häufig vor (Cacchio et al., 2009; Farzan et al., 2010) und die bisherige Datenlage zu den inhibitorischen und exzitatorischen Doppelpuls-paradigmen war inkonklusiv (Boroojerdi et al., 2000; Maeda et al., 2002; Wassermann, 2002; Ngomo et al., 2012). Häufig wurden dabei Frauen aufgrund der hormonellen Schwankungen und deren möglichen Einfluss auf die kortikale Erregbarkeit gänzlich von den Studien ausgeschlossen. Ein Vergleich der zuletzt häufiger verwendeten automatisierten Analyse der CSP mit der visuellen Analyse selbiger und deren Retest-Reliabilitäten ist uns zudem nicht bekannt.

Für die vorliegende Untersuchung (n=93, 36 (38.7%) weiblich) wurden potenzielle Einfluss-faktoren auf die Erregbarkeit, wie das Alter, die Händigkeit der Probanden, und die Tageszeit der Messung konstant gehalten (Maeda et al., 2002; Wassermann, 2002; Pfutze et al., 2007; Sale et al., 2007; McGregor et al., 2012). Frauen wurden nur während der follikulären Phase des menstruellen Zyklus eingeschlossen. Es wurden nur Rechtshänder, gemessen mittels Edinburgh Handedness Inventar (Oldfield, 1971) (EHI≥80), eingeschlossen. Die Probanden waren im Mittel 23.7 ± 3.4 Jahre alt.

Die TMS-Messungen fanden mit einem mittleren Retest Abstand von 34.0 ± 25.6 Tagen (Mittelwert ± Standardabweichung) im gleichen Labor statt. Die Untersuchungen wurden zwischen 7 Uhr morgens und 14 Uhr mittags durchgeführt.

In einer multivariaten Varianzanalyse fanden wir keinen systematischen Unterschied der TMS-Parameter zwischen den beiden Untersuchungszeitpunkten ($p>.1$). Es zeigte sich jedoch für die RMT ein Untersuchereffekt. Einer der Untersucher maß eine höhere RMT als die anderen ($p=.002$). Die anderen Parameter waren nicht untersucherabhängig. Eine Normalisierung der Daten brachte ein ähnliches Ergebnis.

Die Retest-Reliabilität zeigte sich besonders gut für TR (jeweils Korrelationskoeffizient nach Pearson; r=.880) und die RMT (r=.826). Die beiden Messungen der Doppelpulsparadigmen korrelierten weniger hoch miteinander (ICF: r=-.159; SICI: r=.383). Die visuell und die automatisch gemessene CSP zeigten mittlere Korrelationen (respektive r=.466, r=.486). Die Länge des Retest Intervalls, größer oder kleiner als 28 Tage, beeinflusste die Reliabilität nicht.

Betrachtet man die Reliabilitäten der verschiedenen Untersucher sind ebenfalls der TR und die RMT die reliabelsten Parameter. Die Doppelpulsparadigmen zeigten auch bei den einzelnen Untersuchern die schwächsten Korrelationen. Die Wiederholbarkeit der CSP lag bezüglich ihrer Reliabilität dazwischen.

Selten fand aufgrund von organisatorischen Gründen ein Untersucherwechsel zum zweiten Zeitpunkt statt (n=5). Die Reliabilitäten, insbesondere der CSP, nahmen dabei deutlich ab. Dieses geschah sowohl in der visuellen als auch der automatisierten Analyse, so dass hier eher die Messgüte an sich und nicht die Auswertung ein konfundierender Faktor zu sein scheint.

Wir fanden für Frauen und Männer ähnliche Retest-Reliabilitäten, die jeweils denen der Gesamtgruppe ähnelten.

Zusammenfassend zeigte die Studie, dass die TMS ein reliables Verfahren zur Erfassung der kortikalen Erregbarkeit ist. Die Durchführung der TMS durch denselben Untersucher ist in longitudinalen Messungen von Wichtigkeit. Die automatisierte Analyse der CSP zeigte eine nur leicht bessere Reliabilität als deren visuelles Pendant. Die Daten können im Weiteren zur Studienplanung inklusive Fallzahlschätzung und zur Vorhersage von Veränderungen herangezogen werden.

8. Diskussion der Studien und offene Fragen

Zur Messung der kortikalen Erregbarkeit zeigt sich die TMS sowohl in den von der Verfasserin durchgeführten Studien als auch nach der Literatur als ein geeignetes Instrument. Der Einsatzbereich umfasst dabei u.a. klinische Fallstudien und die Untersuchung stoffwechselbedingter und genetischer Faktoren.

Die Ergebnisse der durch die Verfasserin durchgeführten Studien werden nachfolgend besprochen und bezüglich ihrer Berücksichtigung für die Methode der TMS diskutiert.

Die Studie bei pHPT Patienten zeigte keine kalzium-abhängige Änderung der kortikalen Erregbarkeit im Vergleich prä- zu postoperativ. Möglicherweise hatten die kleine Gruppengröße und die aufgrund von organisatorischen Gründen zeitlich variable Nachuntersuchung einen negativen Einfluss. Um dieses zu klären, sollten, neben einer größeren pHPT-Gruppe, in einer nachfolgenden Studie auch hypokalzäme Patienten eingeschlossen werden.

Um (pathologische) Veränderungen der Exzitabilität differenziell zu betrachten ist die Messung und Auswertung der verschiedenen TMS-Parameter (z.B. RMT, CSP) unerlässlich und eine Einzelbetrachtung meist nicht sinnvoll. Nur so konnten wir durch die Änderung der CSP, bei stabiler RMT, die spezifische Modifikation der interneuronalen Aktivität durch den *SCN1A* Polymorphismus rs3812718 beweisen.

Studien zum Effekt von Interventionen können durch Verwendung von Messwiederholungen, wie in o.g. Studien gezeigt, reliabel durchgeführt werden. Der eingereichte Artikel zur Bestimmung der Reliabilität ist dabei aufgrund der großen Teilnehmerzahl gesunder Probanden bedeutend. Die Retest-Reliabilität ist dabei für einige der Parameter höher als für andere. Insbesondere die Ruhemotorschwelle (RMT) scheint intraindividuell sehr stabil. Die Wiederholbarkeit von Messungen der exzitatorischen und inhibitorischen Parameter bei Doppelpulsstimulation (SICI und ICF) wurde in der Literatur bereits kontrovers diskutiert und in unserer Studie als weniger reliabel bestätigt. Die automatisierte Auswertung der Parameter, hier nachgewiesen für die CSP, ist bei geübten Untersuchern der visuellen

Analyse nicht klar überlegen, kann jedoch zur Kontrolle und aus Gründen der besseren Vergleichbarkeit der Ergebnisse longitudinaler Studien ergänzend angewandt werden.

Die Untersuchung von Männern und Frauen erscheint für pharmakologische Studien wichtig für die Bestimmung der Effekte auf die Exzitabilität des Kortex und der Nebenwirkungen, da sonst mögliche geschlechtsspezifische Unterschiede unerkannt bleiben. Viele TMS-Studien schließen bislang eher männliche Teilnehmer ein, um konfundierende Faktoren, wie hormonelle Imbalanzen, so gering wie möglich zu halten. Eine bessere Vergleichbarkeit der Ergebnisse zwischen den beiden Geschlechtern ist aber durch eine bei allen Teilnehmerinnen angewandte feste Definition eines Untersuchungszeitpunktes im menstruellen Zyklus möglich. Die Wiederholbarkeit der TMS-Messungen ist dann, wie in unserer Studie gezeigt, für beide Geschlechter gleich stabil und ergibt keine geschlechts-spezifischen Unterschiede.

8.1 Limitationen

Aufgrund der speziellen Fragestellungen der durchgeführten Studien und deren Umsetzung, u.a. in homogenen Stichproben und festen zeitlichen Protokollen, wurde bewusst auf die Berücksichtigung einiger Aspekte verzichtet. Nichtsdestotrotz sind diese möglicherweise von Relevanz und sollen daher nachfolgend erläutert werden.

Die Gruppengröße in der pHPT-Studie zu Kalziumveränderungen war klein und die Studie tendenziell unterpowert. Das Ergänzen zweier Kontrollgruppen, mit anderen Operationen (z.B. Schilddrüsen-OP) und ohne Operation, hätte spezifischere Aussagen zugelassen. Für einen Rückschluss auf den Wirkmechanismus des Kalzium in Bezug auf die kortikale Erregbarkeit wäre zudem ein hypokalzämes Patientenkollektiv interessant.

Die Untersuchung des *SCN1A*-Gens bei Gesunden und deren differenzielle Veränderung aufgrund der Genvarianten zeigen, dass die kortikale Erregbarkeit vielen Einflussfaktoren unterliegt. Mögliche andere genetische Einflüsse auf die kortikale Erregbarkeit außerhalb des *SCN1A*-Gens wurden in unserer Studie nicht untersucht, sind aber, ebenfalls wahrscheinlich. Da unsere *SCN1A*-Studie in der Fallzahl nur auf den genannten Polymorphismus ausgelegt

war, sind die Verwertungen anderer genetischer Varianten nur begrenzt möglich und deren Einfluss nicht ausgeschlossen. Weitere Studien werden hier Nachweise zu genetisch determinierten Exzitabilitätsänderungen erbringen müssen.

In der durch uns durchgeführten Studie wurden zudem nur die beiden homozygoten Varianten des Polymorphismus als äußere Extreme in vorherigen Studien (Tate et al., 2005; Tate et al., 2006) eingeschlossen, so dass keine Aussage über die heterozygote Variante getroffen werden kann.

Durch den Einschluss von lediglich streng rechtshändigen Probanden kann keine Aussage zum Effekt der Händigkeit oder der Hemisphärendominanz bezüglich einer Retest-Reliabilität gemacht werden.

Folgestudien zur Untersucherabhängigkeit der TMS sind nötig, da die eingeflossenen Studien keine Aussagen über Interrater-Reliabilitäten möglich machten. Teilnehmerstarke Studien sind meist nur durch mehrere Untersucher organisatorisch durchführbar, so dass gerade hier noch entsprechende Nachweise zur Vergleichbarkeit der Daten ergänzt werden sollten. Abhängig hiervon sollte dann gegebenenfalls der Einfluss des Untersuchers als mögliche Störvariable in die Auswertung der Ergebnisse einfließen.

Ungeklärt bleibt die Frage, ob spezielle Veränderungen in den gemessenen Parametern zu bestimmten Zeitpunkten des hormonellen Zyklus auftreten und in welcher Form sich diese auf die Erregbarkeit des Gehirns auswirken. Hier konnte unsere Studie aufgrund des vordefinierten Messzeitpunktes keine Hinweise liefern, weshalb wir eine Abhängigkeit von hormonellen Veränderungen letztlich nicht belegen können. Nur größere longitudinale Studien bei gesunden Frauen zu unterschiedlichen Zeitpunkten im Zyklus und deren Vergleich ohne anderweitige experimentelle Variierung können dieses untersuchen.

Die geringen Reliabilitäten der inhibitorischen und exzitatorischen kortikalen Erregungs-parameter (SICI und ICF) wurden nur mit je einem Interstimulusintervall (ISI) bestimmt.

Möglicherweise könnte das Ergänzen von anderen ISI (z.B. 5ms und 15ms) die Reliabilität verbessern.

Durch die vorgelegten Arbeiten konnte der mögliche Einfluss des Alters auf die kortikale Erregbarkeit nicht festgestellt werden, da es sich jeweils um Querschnittsstudien und meist junge Erwachsene als Probanden handelte. Neben den absoluten Meßwerten könnten auch die Reliabilitäten durch das Alter der Probanden beeinflusst werden. Longitudinale Untersuchungsdesigns sind in künftigen Studien zu wählen, um verlässliche Aussagen zum Alter zu treffen.

8.2 Ausblick

Die durchgeführten Studien der Verfasserin belegen, dass die TMS ein für verschiedene Forschungsfragen einsetzbares, reliables Untersuchungsverfahren ist, welches durch seine nicht-invasive Technik besticht. Zukünftig ist ein vermehrter Einsatz von navigierten TMS-Verfahren, zum Beispiel mittels Co-Registrierung von MRT-Datensätzen, vielversprechend, um non-invasive Verfahren der Deaktivierung (TMS) und Aktivierung (fMRT, funktionelle Magnetresonanztomographie) miteinander zu vergleichen. Ebenso sind Kombinationen mit EEG (Elektroenzephalogramm) vielversprechend, um mögliche kognitive, während der Untersuchung veränderbare, Einflussfaktoren auf die kortikale Erregbarkeit, wie zum Beispiel Wachheit und/oder Aufmerksamkeit, zu monitoren. Durch eine navigierte TMS kann zusätzlich die Konstanz der Spulenposition über die Untersuchungen besser geprüft werden und so die Untersuchungsgüte erhöht werden. Um neben genetischen Einflüssen auf die kortikale Erregbarkeit auch mögliche, ggf. dadurch bedingte, (mikro-) strukturelle Hirnveränderung und deren Einfluss bei geänderter kortikaler Erregbarkeit nachzuweisen, werden Korrelationsstudien zwischen TMS und MRT künftig häufiger zum Einsatz kommen.

9. Zusammenfassung

Die transkranielle Magnetstimulation (TMS) ist ein Verfahren zur Messung der kortikalen Erregbarkeit beim Menschen. Letztere ist durch verschiedene Einflüsse veränderbar und wird in pharmakologischen Studien zur Bestimmung eines Effektprofils, aber auch zu Therapiezwecken, eingesetzt. Verschiedene neurologische Erkrankungen gehen dabei mit einer veränderten kortikalen Erregbarkeit einher. Bei Epilepsien sind je nach Syndrom verschiedene Parameter sowohl der Inhibition als auch der Exzitation verändert. Der Einfluss von Antikonvulsiva als zentralnervös wirksame Substanzen zum Erreichen einer Anfallsfreiheit wurde untersucht.

Die vorliegenden Arbeiten befassten sich einerseits mit stoffwechselbedingten und genetischen Einflussfaktoren auf die mittels TMS gemessene kortikale Erregbarkeit sowie der Retest- Reliabilität, d.h. der Güte der Wiederholbarkeit der TMS.

Die prospektive Untersuchung von Patienten mit einer Hyperkalzämie im Rahmen einer endokrinologischen Erkrankung (primärer Hyperparathyroidismus) konnte keine Veränderung der Exzitabilität durch einen geänderten Kalziumspiegel nach erfolgreicher Operation nachweisen.

In einer weiteren Studie konnte bei gesunden Probanden mit einem Genpolymorphismus des *SCN1A*-Gens, ein differenzieller Einfluss desselbigen auf einen inhibitorischen Parameter, nach Einnahme des Natriumkanalblockers Carbamazepin, nachgewiesen werden. Die TMS kann hier einen Beitrag zur Klärung pharmakogenetischer Einflüsse leisten.

Die Messgüte der TMS, im Sinne einer guten Wiederholbarkeit, ist für die meisten Einzel- und Doppelpuls-Parameter, bei einem gut definierten Kollektiv von gesunden Probanden unter Einbeziehung von weiblichen Studienteilnehmern in einer anderen eingegangenen Studie, objektiviert worden.

Die TMS ist als non-invasives Verfahren zur Messung der kortikalen Erregbarkeit ein für viele Bereiche einsetzbares, robustes Instrument, welches vor allem in Kombination mit weiteren

Messtechniken als wertvolles Instrument für die neurophysiologische Forschung eingesetzt werden kann.

10. Abkürzungsverzeichnis

AED	Antiepileptikum *Antiepileptic drug*
ANCOVA	Univariate Varianzanalyse
Ca^{2+}	Kalzium
CBZ	Carbamazepin
CSP	Kortikale Innervationsstille *cortical silent period*
EEG	Elektorenzephalogramm
EKG	Elektrokardiogramm
EMG	Elektromyographie
fMRT	funktionelle Magnetresonanztomographie
GABA	Gamma-Amino-Buttersäure
ICF	Intraktortikale Bahnung *Intracortical facilitation*
IGE	Idiopathisch generalisierte Epilepsie
ILAE	*International League Against Epilepsy*
ISI	Interstimulus Intervall
JME	Juvenile Myoklonische Epilepsie
LCM	Lacosamid
LICI	Späte Intrakortikale Hemmung *Late intracortical inhibition*
LEV	Levetiracetam
LTG	Lamotrigin
MANCOVA	Multivariate Varianzanalyse
MEP	Motorisch evoziertes Potenzial
PHT	Phenytoin
pHPT	Primärer Hyperparathyroidismus
PTx	Parathyroidektomie
RMT	Ruhemotorschwelle
rTMS	Repetitive TMS
SICI	Frühe Intrakortikale Hemmung *Short intracotrical inhibition*
TMS	Transkranielle Magnetstimulation
TPM	Topiramat
TR	Testreiz

11. Referenzen

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12. Lebenslauf

Die Seiten 44- 46 (Lebenslauf) enthalten persönliche Daten. Sie sind deshalb nicht Bestandteil der Online-Veröffentlichung.

Anhang

Nachdrucke der Publikationen

Clinical Study | A Hermsen and others | Cortical excitability in hyperparathyroidism | 170:2 | 201–209

Perioperative changes in cortical excitability, mood, and quality of life in patients with primary hyperparathyroidism: a pilot study using transcranial magnetic stimulation

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Abstract

Objective: Serum calcium (Ca^{2+}) and parathyroid hormone (PTH), amongst others, modify cortical excitability. Alterations in cortical excitability were shown in patients with epilepsy as well as hyper- or hypoparathyroidism. In patients with primary hyperparathyroidism (pHPT), preoperative elevated serum calcium and parathyroidectomy (PTx) may affect mood and quality of life. We hypothesized that perioperative changes in Ca^{2+} and PTH in pHPT will affect cortical excitability and improve subjective health.

Design and methods: Transcranial magnetic stimulation (TMS) was performed before and after surgery in 15 pHPT patients. We measured resting motor threshold, cortical silent period (CSP), short intracortical inhibition, and intracortical facilitation. Health questionnaires were administered before, 1 day and 6 months after PTx, along with the disease-specific Pasieka's parathyroid assessment of symptoms (PAS), which was, to our knowledge, its first use in German.

Results: Surgery was successful in all patients. TMS-measurements remained unchanged when analyzing all patients in this pilot study. Postoperatively, depression declined ($P=0.05$) and quality of life improved significantly ($P=0.001$) in the SF-36-subscales: vitality, social functioning, mental health and subjective health transition (*post-hoc* analysis). The PAS proved early relief of disease-specific symptoms ($P<0.001$).

Conclusions: We found unchanged cortical excitability comparing pre- and post-PTx in this pilot study. Mood and quality of life improved postoperatively. The German PAS is valuable in detecting disease-specific changes early after PTx.

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Introduction

In humans, 99% of the mineral calcium is found in the bones and teeth. The remaining 1%, found in extracellular spaces, has a major influence on the nervous system, and as an intracellular transmitter can modulate ion channel functions throughout the body (1). Calcium metabolism is mainly regulated by parathyroid hormone (PTH), where elevated serum calcium (Ca^{2+}) levels result in decreased release of PTH and *vice versa*.

Intracellular calcium concentration plays a role in the induction and maintenance of acquired epilepsy in status epilepticus (SE), stroke, and traumatic brain injury (2, 3, 4). These conditions may result in glutamate excitotoxicity and is accompanied by an intracellular increase in calcium concentration levels, resulting in overstimulation of calcium pathways and necrosis (2, 3, 5). Surviving neurons that are exposed to these altered Ca^{2+} levels are prone to

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develop and maintain epileptogenicity (5, 6). Correspondingly, voltage-gated calcium channels have been proposed as a target for antiepileptic drugs in preclinical studies (7).

Clinical evidence that altered Ca^{2+} levels result in changes in cortical excitability has been provided by different case series in hyperparathyroidism (HPT) (8, 9, 10). Primary HPT (pHPT) consists of continually elevated serum calcium and PTH levels (11), mostly due to a solitary parathyroid adenoma (1, 11, 12). The only curative treatment for pHPT is the resection of the suspected solitary adenoma(s) or hyperplastic glands (parathyroidectomy (PTx)) to reestablish normocalcemia which may lead to improved quality of life (13, 14).

Several case reports show higher amplitudes in somatosensory-evoked potentials (8), electro encephalography (EEG)-alterations, reversible vasoconstriction, and epileptic seizures (9, 15, 16) or even (nonconvulsive) SE (10) in patients with hypercalcemia. The primary influence of serum calcium, however, remained controversial in earlier papers (17, 18). A recent study has delivered repetitive transcranial magnetic stimulation (TMS) and found that both acute and chronic hypercalcemia, as tested respectively in pHPT patients and healthy controls by administering infusions of calcium, modified short-term synaptic plasticity (19).

On the other hand, cases of epileptic seizures in hypocalcemia have also been reported (20, 21, 22, 23, 24) and in one neonatal patient seizures were treated by i.v. calcium administration (25).

Cortical excitability can be accurately measured by TMS. Currently, to our knowledge, only one study has investigated the cortical excitability related to changes in PTx-associated calcium in a predominately conservatively treated group of patients with pHPT (19).

Some studies showed that a successful PTx can have positive effects on different domains of quality of life such as increased energy levels and improved physical as well as emotional well-being (26, 27, 28). However, other studies found no evidence of a positive change in depression scales when comparing PTx patients with patients who underwent orthopedic surgery (29). Concerning asymptomatic pHPT patients, some randomized control studies found moderate positive effects of operation vs medical care on quality of life (30, 31) while others did not (32).

As the role of calcium in cortical excitability is yet to be clarified in humans, pHPT-patients who underwent surgery served as a model for rapid changes in serum calcium in this pilot study. When we investigated the patients, most of them were symptomatic before and after PTx with TMS and administered several mood and quality of life questionnaires up to 6-months' follow-up to identify perioperative changes. Additionally, to our knowledge, this was the first time that a German version of Pasieka's parathyroid assessment of symptoms (PAS) score was administered.

As calcium seems to influence cortical excitability in several ways, we hypothesized that based on the expected transition from hyper- to normocalcemia, postoperative cortical excitability, as measured by TMS, would significantly decrease compared with preoperative measurements. Furthermore, postoperative normocalcemia should lead to an improved quality of life and mood.

Subjects and methods

Patients

Twenty right-handed patients (nine males and 11 females) diagnosed with pHPT and scheduled for PTx at the Department of Surgery of the University Hospital Marburg were recruited. They had no history of acute or chronic neurological or psychiatric disease and were not taking medication for CNS indications.

Two female patients withdrew from the study due to TMS intolerance and three patients were excluded because of lack of EMG response, sulcus ulnaris syndrome diagnosis, or postponed parathyroid surgery.

TMS data from the remaining 15 patients (seven males and eight females, median age 55.0 years, range 22–73 years) were obtained before and after PTx. Gender (early: 5F, 6M vs late: 3F, 2M, $P=0.742$) and age (early: 55.0 (44.0–69.0) vs 59.0 (47.5–70.0), $P=0.462$) were equally distributed in the early and late groups. Twelve patients presented with typical symptoms and three were asymptomatic (see Table 1). For the asymptomatic group, the NIH criteria for operation in asymptomatic pHPT patients were met (33).

TMS measurements after the operation were undertaken more than 30 h after PTx to adjust to the half time of the typically used narcotics and to eliminate possible effects of anesthesia. Those who were only found to be dosage dependent, as far as we know, were only studied during intraoperative monitoring and not after anesthesia (34).

The study was approved by the Local Ethics Committee of Philipps University of Marburg and written informed consent was obtained from all patients before participation.

Table 1 Patient characteristics.

Patient no.	Age	Gender	Symptomatic/ asymptomatic	Symptoms
3	22	M	Symptomatic	Recurring renal colic
4	46	M	Symptomatic	Kidney stones, renal colic
7	72	M	Symptomatic	Renal colic
8	55	F	Symptomatic	Stomach trouble, osteoporosis
9	66	M	Symptomatic	Acute pancreatitis, Ca >0.2 mmol above norm
15	59	F	Symptomatic	Bone pain, renal colic
17	40	F	Symptomatic	Depression, explicit operation wish
23	44	F	Symptomatic	Bone pain, tiredness, depression
27	58	M	Symptomatic	Pancreatitis
33	69	F	Symptomatic	Bone pain
54	49	M	Asymptomatic	–
60	73	F	Symptomatic	Bone pain
69	71	F	Asymptomatic	Fulfills NIH criteria[a] for asymptomatic pHPT with Ca >0.2 mmol above norm
70	37	F	Asymptomatic	Fulfills NIH criteria[a] for asymptomatic pHPT with Ca >0.2 mmol above norm
84	52	M	Symptomatic	Fatigue/depression, polyuria

[a]As defined in the article of Bilezikian *et al.* (33).

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Transcranial magnetic stimulation

TMS was administered on the day before surgery (−1±0 days) as well as after a median of 3 days (1 day, 25th quartile–142 days, 75th quartile) for all subjects. Subjects were comfortably seated in an armchair with their head fixed in a custom plastic foam headrest. TMS was delivered through a focal figure-of-eight-shaped magnetic coil (70 mm external loop diameter) connected to two Magstim 200 magnetic stimulators via a BiStim-module (all Magstim, Whitland, Dyfed, UK). The coil was placed flat on the head over the left motor cortex, approximately at an angle of 45° to the sagittal plane, inducing a current in the brain region roughly perpendicular to the central sulcus, flowing from posterior to anterior, as this has been reported to be the most effective way to activate the corticospinal system transsynaptically (35). Motor-evoked potentials (MEP) were recorded using surface EMG Ag/AgCl electrodes placed over the right abductor digiti minimi muscle using a belly-tendon montage. The raw signal was amplified, filtered (10–20 kHz), and recorded with a PC using a commercially available data-collection and averaging program (Magnetix, Centre of Sensorimotor Research, Munich, Germany) for offline analysis. The optimal coil placement was determined by recording MEP while varying the coil position. The coil position leading to the highest peak-to-peak amplitude of the MEP ('hot spot') was marked with a semi-permanent pen directly on the scalp to ensure accurate coil positioning during testing.

All sessions followed a fixed sequence of TMS measurements: first, the test stimulus (TS) and resting motor threshold (RMT), and then the paired-pulse parameters, short intracortical inhibition (SICI) and intracortical facilitation (ICF), were obtained in a random order. In all paired-pulse TMS procedures, the interval between trials was randomly changed between 4 and 6 s; in single-pulse procedures the inter-trial interval was 5 s. The protocol was concluded with determination of the cortical silent period (CSP).

While RMT and ICF are excitatory parameters, SICI and CSP determine the inhibitory phenomena. All of those show changes at different neurotransmitter systems (36). Voltage-gated sodium channel mechanisms influence the RMT. SICI and ICF are influenced by glutamatergic and the $GABA_A$ systems in the cortex. The CSP shows inhibitory $GABA_B$-mediated long-term mechanisms, and these are probably also responsible for late intracortical inhibition (LICI).

TMS parameters

TMS parameters were specified as follows:

i) The RMT was defined as the lowest stimulator output intensity required to induce MEP peak-to-peak amplitude >50 μV in at least five of ten consecutive trials. Complete muscle relaxation was monitored via audio-visual feedback. A step-by-step intensity resolution of the maximal stimulator output was used for the determination of the individual RMT using the maximum likelihood threshold hunting (MLTH) procedure for TMS (© Dr Friedemann Awiszus, Magdeburg (37)).

ii) SICI and ICF were obtained with paired-pulse TMS. A conditioning and a TS were applied with different fixed interstimulus intervals (ISI). The conditioning stimulus was set to an intensity of 75% of the RMT, as this does not produce changes in excitability in the spinal cord (38, 39). The intensity of the following suprathreshold TS was adjusted to produce MEPs of ~1.5 mV peak-to-peak amplitude if delivered without a preceding conditioning stimulus (TS). SICI was obtained at short ISIs of 2 and 3 ms, leading to a decreased MEP compared with an MEP induced by a nonconditioned TS. ICF was obtained using ISIs of 10 and 15 ms, leading to an increased MEP (35, 36, 39, 40, 41). Owing to good correlation of the inhibitory ISIs of 2 and 3 ms (pre: $\rho=0.696$, $P=0.004$ and post: $\rho=0.892$, $P<0.001$), we computed one variable (ICI) and did the same for ISIs of 10 and 15 ms (pre: $\rho=0.948$, $P<0.001$ and post: $\rho=0.876$, $P<0.001$), which are measures of ICF. LICI was tested at ISI of 150 ms and two equal stimuli of 150% RMT were delivered. Fifteen trials of single nonconditioned test stimuli and 15 paired stimuli of each ISI, generated in random order by the computer software, were recorded. The average of the 15 trials was used to define the amplitude of the peak-to-peak MEP for each condition. The conditioned response was defined as the mean amplitude of the conditioned responses belonging to each ISI, expressed as the percentage of the mean amplitude of the unconditioned test response. The CSP was measured during 20 trials at a stimulus intensity of 110% of the RMT. Participants were instructed to hold a voluntary muscle contraction of ~30% of their maximal force, controlled by audio-visual feedback. CSP duration was determined offline in two ways. For visually guided analysis, the CSP duration was defined as the time from TMS stimulus artefact to the first reoccurrence of voluntary EMG activity exceeding 25% of muscle activity before the stimulus. This was always determined by the same investigator to minimize variability. Duration was determined offline in the Magnetix program.

One value per person and session was calculated by averaging separately for RMT, SICI, ICF, LICI, and CSP.

Emotional well-being, quality of life

Questionnaires on subjective health and mood status were filled in by the patients on the day before operation, 1 day after operation and half a year after operation. The Beck Depression Inventory (BDI-II) (42) measures levels of depression on a four-point Likert scale for 21 statements. The patient is asked to mark the statement that best fits his mood for the past 2 weeks (0–3 points). The sum of scores determines the degree of depression, with a score equal to or above 14 being clinically relevant (maximum 63 points). The Short Form 36 Health Survey (SF-36 (43, 44)) is an instrument used to determine individual nondisease-specific health in patients. At the level of raw scores, higher scores indicate more impairment except for self-reported health transition, whereas higher values indicate negative health development in the last year. Several subscores and two components, mental (mental component score (MCS)) and physical health (physical component score (PCS)) (45), can be measured.

Pasieka's PAS score (46) was applied to detect specific symptoms in the pHPT patient group. The PAS measures 13 aspects of parathyroidism that are disease-specific (e.g. bone pain, mood alteration, degree of weakness, and being forgetful) on a visual analog scale (47) with a maximum score of 1300. Lower scores refer to less disturbing symptoms. Additionally, two scores on quality of life and well-being can be scored separately (maximum each ten points). As no German version of the PAS was readily available, items were translated by a native English speaker.

Clinical parameters

The serum calcium and PTH levels, as well as clinical data including medication and symptoms of hyper- and hypocalcemia, were determined on the day of admission to the ward and following PTx. Grip force was measured after each TMS session. Each patient squeezed a hydraulic hand dynamometer (Saehan Corp., Masan, Korea) at maximum power with their dominant hand.

The F-waves ($n=5$) and nerve conduction velocity ($n=7$) were measured to discover possible peripheral nerve lesions or polyneuropathy and reduce confounding with the results of TMS measurements.

Statistical analysis

The analysis was conducted using PASW Statistics 18–20 (SPSS, IBM Company). Data are expressed as median ± 25th and 75th percentiles or SD. Owing to sample size and skewed data, we chose to use nonparametric statistical tests. The Mann–Whitney U test was applied to independent samples and the Wilcoxon's rank sum test for paired samples. To determine associations between TMS

Table 2 TMS parameters for the entire group ($n=15$), pre- and post-PTx as measured by Wilcoxon's test.

	Pre-PTx	Post-PTx	*P*
RMT	44.0 (42.0–51.0)	45.0 (38.0–54.0)	0.711
ICF	135.8 (113.3–220.1)	137.5 (99.5–174.6)	0.910
SICI	46.8 (35.5–94.7)	49.4 (37.8–70.0)	0.910
LICI	73.1 (52.2–89.0)	76.7 (66.2–88.8)	0.394
CSP	94.9 (74.3–124.4)	100.6 (80.6–113.1)	0.552

PTx, parathyroidectomy; RMT, resting motor threshold (in % of maximal stimulator output); ICF, intracortical facilitation; SICI, short intracortical inhibition; LICI, late intracortical inhibition; CSP, cortical silent period (ms); ICF, ICI, and LICI all as % of unconditioned MEP; numbers are displayed as median (25th–75th quartiles).

parameters and Ca^{2+} and PTH, Spearman's correlation coefficient (ρ) was used.

Multivariate ANOVA (MANOVA) was applied with the three-staged within-subject factor time (pre, post, and 6-months' follow-up) and questionnaire-specific dependent variables. If group variances were nonhomogenous (according to Mauchly's test of sphericity), degrees of freedom were adjusted (Greenhouse-Geisser). When appropriate, either univariate ANOVA or *post-hoc* tests (Bonferroni's adjusted) are reported.

Not all participants returned all questionnaires. This resulted in reduced data (BDI, $n=12$ and SF-36, $n=13$). For determination of PAS changes, we left out one patient due to outlier responses ($n=12$).

Results

Hormone levels and clinical variables

A significant reduction in Ca^{2+} and PTH level was achieved by surgery from pre- to post-measurement (Ca^{2+} pre: 2.8 (2.6–2.9) vs post: 2.3 (2.2–2.5), $P=0.004$; PTH pre: 133.00 (107.00–170.00) vs post: 12.00 (6.75–23.5), $P=0.002$). Postoperatively, Ca^{2+} and PTH were significantly correlated ($r=-0.704$, $P=0.011$). Grip force (33.50 (27.5–43.75) vs 36.00 (27.50–44.00), $P=0.721$) as well as F-waves ($P=0.236$) and nerve conduction velocity ($P=0.144$) remained stable from pre- to post-operation.

Cortical excitability

We found significant correlations between the two measurements for the following TMS parameters: TS ($\rho=0.810$, $P<0.001$), RMT ($\rho=0.649$, $P=0.009$), SICI ($\rho=0.607$, $P=0.016$), and ICF ($\rho=0.504$, $P=056$). Ca^{2+} and PTH, however, showed no consistent correlations with any TMS parameters, either pre- or post-PTx.

There were no significant alterations in the TMS parameters of the entire group when comparing pre- and post-PTx (see Table 2).

Emotion and well-being

Multivariate repeated measure analysis (RM-MANOVA) with the BDI score as the dependent variable and time as the within-subject-factor indicated an overall decline in depressive symptoms over time ($P=0.05$, see Table 3). Univariate analysis revealed that these symptoms remained stable from pre- to immediately post-PTx ($P=0.192$) but that patients were significantly less depressed at 6-months' follow-up compared with pre- and post-measurements ($P=0.018$ and $P=0.039$ respectively).

The analysis of the overall effects of all subscales in the SF-36 revealed a multivariate effect for 'time' ($F(18,28)=3.65$, $\eta^2=0.701$, $P=0.001$). We subsequently determined the significantly altered subscores in univariate analysis. These showed significant improvements in vitality, social functioning, mental health, and subjective health transition ($P<0.001$, $P=0.016$, $P=0.042$, and $P=0.001$ respectively, see Fig. 1). *Post-hoc* analysis clarified that with the exception of mental health, which only changed from post-PTx to 6-months' follow-up, all the other scores improved significantly from pre- to post-PTx and from pre-PTx to 6-months' follow-up, while no significant immediate-pre–post changes were found (see Fig. 1). When separately analyzing the subscales of the PCS and MCS, we found no significant changes across measurements.

Disease-specific PAS score

Analysis revealed a decline in PAS symptoms over time ($n=12$, $P<0.001$). However, univariate analysis showed a decline only from pre- to immediately post-PTx (pre-PTx,

Table 3 Change in BDI and PAS over time.

	Pre-PTx	Post-PTx	6-months FU	*P*
BDI mean ($n=11$)	9.6±5.4	7.6±6.4	5.4±4.8	0.05
PAS sum ($n=12$)	342.6±218.7	271.6±225.9	189.9±173.2	<0.001

BDI, Beck Depression Inventory; PAS, parathyroidect assessment of symptoms scores; pre, 1 day before parathyroidectomy (PTx); post, 1 day after PTx; FU, follow-up; data are displayed as median±S.D., multivariate *P* is given.

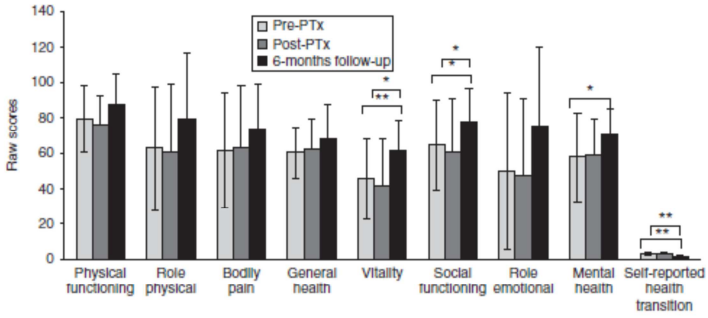

Figure 1

Subscales of SF-36 for pre, post, and 6-months (FU; $n=12$). In *post-hoc* analysis vitality, social functioning, and self-reported health transition differed from pre to FU and post to FU. Mental health changed from post to FU only. Note that higher scores mean more impairment except for self-reported health transition, where fewer points are positive. Median and SD. are displayed. PTx, parathyroidectomy, $^{*}P<0.05$ and $^{**}P<0.001$.

342.58±218.69; post-PTx, 271.58±225.90, $P=0.047$). The PAS furthermore showed strong overall correlations between the immediate post-measurement and the 6-months' follow-up ($\rho=0.983$, $P<0.001$), indicating that long-term postoperative improvement can be predicted by the early postoperative PAS score. The two PAS subscores for quality of life and well-being were also highly correlated with both measurements after the operation ($\rho=0.849$, $P=016$ and $\rho=0.934$, $P=0.002$ respectively).

All questionnaires correlated well in the vast majority of domains at the three times of measurement. This included amongst others very good correlations of the BDI and PAS on all inquiries (pre $\rho=0.778$, $P=0.002$; post $\rho=0.920$, $P=0.000$; and 6 months $\rho=0.862$, $P=0.000$).

Our patient group displayed good to strong correlations between PAS and SF-36 for the following subscales: general health, vitality, social functioning, role emotional, and mental health (see Table 4) but none with all eight subscales and the overall change in health.

We found that calcium correlated after the operation with a decrease in depressive symptoms on BDI ($r=-0.633$, $P=0.027$) and an improvement in PAS well-being ($r=0.809$, $P=0.005$). Several SF-36 scales (role physical, overall health, vitality, emotional well-being) correlated with postoperative calcium ($r=0.670$, $P=0.009$; $r=0.619$, $P=0.024$; $r=0.712$, $P=0.004$; and $r=0.731$, $P=0.003$ respectively). There were no correlations of calcium and any BDI or PAS-score before the operation. For SF-36, we found preoperative correlations with calcium on the subscale physical functioning.

Discussion

This study collected data on perioperative changes in cortical excitability measured by TMS and changes in mood and quality of life following successful PTx and consecutive normalization of serum calcium levels from patients with pHPT. We aimed to understand calcium-dependent alterations by using pHPT as a model for rapid calcium changes in humans. Therefore, cortical

Table 4 Significant correlations between PAS and SF-36 subscales.

	PAS		
	Pre	Post	6 months
SF-36 pre			
General health	0.565*		
Vitality	0.754*		
Social functioning	0.742*		
Role emotional	0.749*		
Mental health	0.844†		
SF-36 post			
General health		0.711*	
Vitality		0.774*	
Social functioning		0.685*	
Role emotional		0.682*	
Mental health		0.757*	
SF-36 6 months			
General health			0.887†
Vitality			0.931†
Social functioning			0.670*
Role emotional			0.758*
Mental health			0.910†

*$P<0.05$ and †$P<0.001$.

excitability was measured pre- and postoperatively in each patient. Changes in mood and quality of life following PTx up to 6-months' follow-up were investigated. This is, to our knowledge, the first study to use a German version of the disease-specific Pasieka's PAS score.

TMS measurement was performed in all patients before PTx to obtain data during a hypercalcemic state and after PTx to collect data on cortical excitability during normocalcemia. Using this within-subject design restricted confounding interindividual factors that arise when comparing different patients. Surgery was successful and all 15 patients who completed the study showed a significant reduction in both Ca^{2+} and PTH levels into normal range postoperatively.

Cortical excitability showed strong correlations among TMS parameters for both sessions, affirming the relatively stable intra-individual cortical excitability. Considering the whole group of patients, we did not find significant alterations in cortical excitability as measured by TMS. At least for hypercalcemia, it has been reported that EEG patterns normalize gradually after treatment with hypocalcemic agents (4). Lacovelli *et al.* (19) in their rTMS study reported only two pHPT patients undergoing PTx that showed unchanged RMT, CSP, and MEP amplitude but improved MEP facilitation after PTx. We could not replicate this finding in a larger cohort.

Changes in patients' overall health as well as mental health were positive after PTx. Promisingly, the PAS, designed as a disease-specific tool, showed a decline from pre- to immediately post-PTx and strong correlations between immediate post-operative measurement and a longer 6-months' follow-up. This therefore underlines the rapid positive impact of the surgical intervention (28) even though, due to our study design, we can't compare with a surgical control-group. The PAS therefore seems an excellent tool during the hospital phase for early prediction of changes in specific symptoms. Ongoing postsurgical recovery or experience of surgery on the first postoperative day might have influenced the unchanged depression scores between pre- and immediately post-operation. When, in a different study, first measurement was held at two weeks, postoperatively depressive symptoms had declined (48). Similarly, our longer follow-up showed a decline from both earlier measurements to 6-months' follow-up and therefore we conclude that depressive symptoms normalize over time. Depressed mood is one of the clinically relevant neuropsychiatric symptoms in pHPT, while the influence of calcium on depressive symptoms remains unclear (29, 49). Like Chiang *et al.* (29), we found a slight and diverse evidence of any dependency of the questionnaires and cortical excitability on calcium levels, while recent research has found a correlation of CA^{2+} and depression, suicidal tendencies and quality of life (48). This might stem from the larger multicenter cohort of Weber *et al.* and should be verified in future studies.

Similar to Mihai & Sadler (27), who showed a good correlation between the well-established SF-36 and the original English PAS, we were able to show a good correlation for both German versions. Our data emphasize that its administration before and immediately after PTx yields reliable insights into specific changes and that long-term follow-up might not be required as the data did not subsequently change. This should be confirmed by prospective trials with a larger number of patients. The disease-specific items and the faster administration time of the latter should promote the use of PAS in surgical settings for quick and objective assessment of perioperative changes in pHPT.

Limitations of our study are the lack of any control group to better distinguish the results as the effects of PTx, unspecific surgery, or disease relief effects. Furthermore, the number of enrolled patients is rather small, which led to slightly underpowered TMS measurements.

Further research should include a larger number of patients with pHPT. Additionally, cortical excitability associated with hypocalcemia should be measured as hypocalcemia, or a rapid change in calcium balance after prolonged hypercalcemia (50) in certain cases, may correlate with a decrease in seizure threshold and is consistent with studies on both rodent hippocampal slices (51) and human case reports (11, 51, 52). This might influence the clinical treatment of an altered calcium balance. A control group should be included, preferably both a nonsurgical as well as surgical group, to differentiate the effects of disease cure, anesthesia, and surgery especially in the mood domain.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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FULL-LENGTH ORIGINAL RESEARCH

A common *SCN1A* splice-site polymorphism modifies the effect of carbamazepine on cortical excitability—A pharmacogenetic transcranial magnetic stimulation study

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Summary

Objective: ***SCN1A*** **encodes the alpha subunit of the voltage-gated sodium channel and plays a crucial role in several epilepsy syndromes. The common *SCN1A* splice-site polymorphism rs3812718 (IVS5N+5 G>A) might contribute to the pathophysiology underlying genetic generalized epilepsies and is associated with electrophysiologic properties of the channel and the effect of sodium-channel blocking antiepileptic drugs. We assessed the effects of the rs3812718 genotype on cortical excitability at baseline and after administration of carbamazepine in order to investigate the mechanism of this association.**

Methods: **Paired-pulse transcranial magnetic stimulation (TMS) was applied in 92 healthy volunteers with the homozygous genotypes AA or GG of rs3812718 at baseline and after application of 400 mg of carbamazepine or placebo in a double-blind, randomized, crossover design. Resting motor threshold (RMT), short interval intracortical inhibition (SICI), intracortical facilitation (ICF), and cortical silent period (CSP) were determined.**

Results: **At baseline there was no significant difference in any TMS parameter. Genotype GG was associated with a higher carbamazepine-induced increase in CSP duration as compared to AA (multivariate analysis of covariance [MANCOVA], $p = 0.013$). An expected significant increase in RMT was genotype independent.**

Significance: **We found that the rs3812718 genotype modifies the effect of carbamazepine on CSP duration (mainly reflecting modulation of γ-aminobutyric acid (GABA)ergic inhibition), but not on RMT (mainly reflecting modulation of voltage-gated sodium channels). This provides evidence that rs3812718 affects the pharmacoresponse to carbamazepine via an effect on GABAergic cortical interneurons. Our results also confirm that TMS is useful to investigate the effect of genetic variants on cortical excitability and pharmacoresponse.**

KEY WORDS: **Transcranial magnetic stimulation, Resting motor threshold, Cortical silent period, Pharmacogenetics, Drug response.**

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The *SCN1A* gene encodes the alpha subunit $Na_v1.1$ of the voltage-gated sodium channel and plays a crucial role in the pathogenesis of several monogenic epilepsy syndromes, including genetic epilepsy with febrile seizures plus (GEFS+) and Dravet syndrome.[1,2] Recent studies suggest that *SCN1A* may also be a susceptibility gene in seizure syndromes with complex inheritance. Schlachter et al.[3] reported that the A allele of a common *SCN1A* splice site polymorphism (IVS5N+5 G>A, dbSNP: rs3812718) was associated with febrile seizures. This was confirmed by some[4] but not all studies.[5,6] A recent genome-wide association study revealed a suggestive association between genetic generalized epilepsies (GGEs) and chromosomal regions encompassing the *SCN1A* gene, thereby underscoring the importance of this gene for GGE.[7]

The genetic findings are supported by data showing that the A allele of rs3812718 leads to reduced expression of the neonatal exon 5N relative to the adult exon 5A.[8,9] which can alter the electrophysiologic properties of $Na_v1.1$ in vitro.[10]

The single nucleotide polymorphism (SNP) rs3812718 was also found to have pharmacogenetic relevance, as an influence of this polymorphism on the response to sodium channel blockers, including carbamazepine, phenytoin, and lamotrigine, in patients with epilepsy was detected by several studies,[8,11–13] whereas the results of other studies are conflicting.[14,15] However, the impact of the polymorphism on the human drug response in human cortical networks has not yet been assessed, leaving the mechanism linking this polymorphism to drug dosage unclear.

Multiparametric transcranial magnetic stimulation (TMS) offers the opportunity to separately examine the excitatory and inhibitory properties of the human cortex and modifying factors in vivo, including the effects of antiepileptic and other drugs.[16–21] We aimed to test two hypotheses with TMS: (1) The SNP rs3812718 is associated with baseline cortical excitability, and (2) rs3812718 is associated with altered excitability after administration of the sodium channel blocker carbamazepine (CBZ) as compared to placebo. Verification of hypothesis 1 would suggest that the common *SCN1A* splice-site SNP rs3812718 might play a role in the pathophysiology of complex epilepsies. Verification of hypothesis 2 would support the consideration that this SNP is of pharmacogenetic relevance.

Subjects and Methods

Subjects

The number of subjects was determined by a sample size calculation. With $\sigma = 2.5$ and a speculated difference of 1.5 between the genotypes on RMT, that were determined as detectable difference, and power of $1-\beta = 80\%$, 46 participants were needed for each genotype group using *t*-tests. Considering the distribution of the genotypes in the general population and the dropout rate, we genotyped 271 healthy volunteers in order to identify volunteers with rs3812718 genotype AA (approximately 25% of the general population) and GG (approximately 22% of the general population). Healthy subjects were recruited from the geographical area served by the Marburg University Hospital. All subjects were of European ancestry.

Main inclusion criteria were the following: right-handedness as determined by the Edinburgh Handedness Inventory (EHI, EHI-score ≥ 80), age 18–60 years, reliable contraception for women (Pearl index < 0.1), the cognitive and physical ability to understand the experimental procedure, willingness to ingest the study medication (CBZ, placebo) and to be investigated by TMS. Main exclusion criteria included neurologic/psychiatric diseases, serious medical conditions such as cancer, cardiac problems and endocrinologic disorders, epileptic seizures, nonepileptic seizures, use of central nervous system (CNS)–active drugs, metal implants in neck or head, as well as pregnancy or lactation.

Subjects were instructed to refrain from smoking or taking CNS-active substances like caffeine for at least 24 h before each TMS investigation.

The study was approved by the local institutional review board (IRB) and the German Federal Institute of Drugs and Medical Devices (BfArM; eudraCT number 2008-003392-40).

Methods

After a detailed explanation of the experimental procedure and providing written informed consent, the *SCN1A* SNP rs3812718 was genotyped on an ABI Prism 7900HT Sequence Detection System (Life Technologies Corporation, Carlsbad, CA, U.S.A.) using a TaqMan SNP Genotyping Assay (Assay ID: C__25982233_10; Life Technologies Corporation). Subjects with the genotypes AA and GG were further investigated using TMS. TMS was performed on two different days at least 2 weeks apart before (baseline) and 5 h after intake of either placebo or 400 mg of CBZ in a randomized (block randomization, permutated block size), crossover, double-blind study design.

Female subjects had a pregnancy test on both days. In addition, all subjects underwent electrocardiography (ECG) before the first TMS session and were excluded from the study if the ECG showed any abnormalities.

Transcranial magnetic stimulation measurements

Transcranial magnetic stimulation (TMS) was delivered through a figure-eight–shaped magnetic coil (external diameter of each loop 9 cm; current in the junction of the coil directed from anterior to posterior). The coil was connected to two magnetic stimulators with a monophasic current waveform (Magstim 200) via a Bistim Module (Magstim; Whitland, Dyfeld, United Kingdom). Maximum stimulus intensity was 2T.

Subjects were seated in an armchair with the head fixed in a plastic foam headrest. The coil was placed flat on the skull at an angle of 45 degrees to the sagittal plane, inducing

a current in the brain approximately perpendicular to the central sulcus, flowing from posterior to anterior. The optimal coil position was determined by recording motor evoked potentials (MEPs) while varying the coil position. The coil position leading to the highest peak-to-peak amplitude of the MEP was marked directly on the scalp to ensure accurate coil repositioning.

In each subject, the left, presumably dominant hemisphere was evaluated. Motor evoked potentials were recorded using surface electromyography (EMG) Ag/AgCl electrodes placed over the right abductor digiti minimi muscle (ADM) in a belly-tendon montage. The raw signal was amplified, filtered (20 Hz–10 kHz) and recorded with a PC using a commercially available data collection and averaging program (Magnetix; Center of Sensorimotor Research, Munich, Germany) for offline analysis.

The TMS parameters resting motor threshold (RMT), short-interval intracortical inhibition (SICI), facilitation (ICF), and cortical stimulation-induced silent period (CSP) were used to investigate motor cortex excitability.

The RMT was defined as the minimal stimulus intensity required to induce a MEP of more than 50 μV peak-to-peak amplitude in at least 5 of 10 consecutive trials. Complete muscle relaxation was monitored via audiovisual feedback. A step width of 1% of maximal stimulator output was used for determination of the RMT. The program MLTH (maximum likelihood threshold hunting procedure for TMS©; Dr. Friedemann Awiszus, Magdeburg, Germany) was used for determination of the RMT.

SICI and ICF were obtained during paired-pulse TMS. A conditioning and a test stimulus were applied with different fixed interstimulus intervals (ISIs). The conditioning stimulus was set to an intensity of 75% of RMT, which produces no excitability changes in the spinal cord.[22] The intensity of the following suprathreshold test stimulus (TS) was adjusted to produce MEPs of approximately 1.5 mV peak-to-peak amplitude if delivered without preceding conditioning stimuli. SICI was obtained at short ISIs of 3 msec, leading to a decreased MEP as compared to an MEP induced by a nonconditioned test stimulus. ICF was obtained using ISIs of 10 msec, leading to an increased MEP.[20,22] Fifteen trials of single, nonconditioned test stimuli and 15 paired stimuli of each ISI were recorded, each generated in random order by the software. The average of the 15 trials was used to define the amplitude of the peak-to-peak MEP for each condition. The conditioned response was defined as the mean amplitude of the conditioned responses belonging to each ISI, expressed as percentage of the mean amplitude of the unconditioned test response. For better comparability, this percentage was subtracted from 100% for SICI (SICI: 100% − [conditioned response/unconditioned response × 100%]; ICF: conditioned response/unconditioned response × 100% as previously suggested).[23]

The CSP was measured in 20 trials at a stimulus intensity of 110% of the RMT. The subjects were instructed to hold a voluntary muscle contraction of approximately 30% of the maximal force, monitored by audiovisual feedback. The CSP duration was defined in individual trials as the time interval from the beginning of the stimulus-induced MEP to the first recurrence of voluntary EMG activity displayed.

To reduce the duration of the TMS session necessary for determination of RMT, TS, SICI, ICF, and CSP and the risk of head movements, SICI and ICF measures were determined only at a stimulus intensity of 75% of RMT and the CSP at a stimulus intensity of 110% of the RMT.

One single value per person and test session was calculated by averaging separately for RMT, TS, SICI, ICF, and CSP.

Five hours after intake of either placebo or CBZ, the serum level of CBZ was determined in order to account for possible differences between the two genotypes. In addition, subjects were asked to complete a questionnaire concerning the side effects of the study drug (Table S1).

Female subjects were investigated in the follicular phase to exclude confounding of the results by hormonal fluctuations in the course of the menstrual cycle.[24,25]

Statistical analysis

Statistical analysis was computed with IBM SPSS Statistics 20 (SPSS; IBM Company, Chicago, IL, U.S.A.). Chi-square tests were applied to categorical variables and *t*-tests for independent samples to metric variables, respectively. *t*-Tests for paired samples were used to compare overall treatment effects of CBZ versus placebo irrespective of the genotype. Baseline measurements from sessions 1 and 2 were averaged individually for further analysis.

We included the two TMS parameters RMT (reflecting mainly modulation of voltage-gated sodium channels) and CSP (reflecting mainly modulation of GABA-ergic inhibition) in the analysis of a possible selective effect of CBZ for the genotypes AA and GG, as these two parameters were previously shown to be influenced by CBZ.[16,18,26] A multivariate analysis of covariance (MANCOVA) with the two-stage-factor genotype (AA vs. GG), the differences in change in RMT and CSP from baseline to postmedication (CBZ-PL) as dependent variables, and the covariates gender, CBZ-level, and TS after intake of CBZ were computed. TS, SICI, and ICF were evaluated in an exploratory analysis using *t*-tests for independent samples.

Pearson's correlation coefficient was determined for the association between CBZ level, gender, and TMS parameters as well as side effects. Statistical significance was set to $p < 0.05$ (two-tailed).

Results

Subject characteristics

Of the 271 subjects who underwent genotyping, 140 (51%) had either the AA (77 subjects, 28.4%) or the GG (63 subjects, 23.2%) genotype of rs3812718. The distribution of

the genotypes did not deviate significantly from that predicted by the Hardy-Weinberg equilibrium ($p = 0.61$).

Overall dropout or removal from the study was 34.3% (48 of the 140 subjects with genotype AA or GG). Of the volunteers, 39 (16 GG, 23 AA) were not willing to complete the study after genotyping, and one additional subject (genotype GG) dropped out after the placebo visit. In addition, eight volunteers (three GG, five AA) were excluded from the statistical analysis (two due to technical problems during TMS measurements, one due to perinatal hypoxia not reported earlier, and five due to missing CBZ levels). The remaining sample comprised 92 healthy volunteers (49 with genotype AA and 43 with genotype GG).

Demographic data are displayed in Table 1. The genotype groups did not differ in age or gender frequency. Randomization was successful in distribution of treatment order, and the CBZ levels were comparable in the two groups.

Baseline differences in cortical excitability

Baseline measures of cortical excitability in both conditions were averaged and revealed no group difference in RMT between genotype GG and AA ($p = 0.402$, Table 2). Likewise, there were no statistically relevant dissimilarities between the genotypes on mean baseline TS, SICI, and ICF or CSP (all $p > 0.05$, Table 2). The mean baseline RMT was higher for women than for men (46.68 ± 9.09 vs. 42.75 ± 7.07; $T = -2.33$; $p = 0.022$). The genotype-dependent TMS results at baseline and after administration of CBZ and placebo are listed in Table 3.

Overall effect of CBZ

Analysis of the overall effect of CBZ for all subjects revealed a greater increase in RMT from baseline values following administration of CBZ as compared to placebo (RMT: $t(90) = -3.26$, $p = 0.002$, Table 4). In addition, TS

Table 1. Demographic data of the two genotypes AA and GG; numbers or mean (SD)

Variable	AA (n = 49)	GG (n = 43)	p-Value[a,b]
Gender (m/f)	25/24	27/16	0.256
Age	23.5 (2.7)	24.4 (3.9)	0.224
Randomization (PL-CBZ/CBZ-PL)	27/22	17/26	0.136
CBZ-level (mg/L)	4.5 (1.2)	4.6 (1.0)	0.545

SD, standard deviation; m, male; f, female; PL, placebo; CBZ, carbamazepine.
[a]t-Test for independent samples.
[b]Chi-square test.

Table 2. Mean (SD) of TMS measures at baseline between genotype AA and GG

Variable	AA (n = 49)	GG (n = 43)	p-Value[a]
TS	57.57 (8.36)	55.28 (7.59)	0.174
RMT	45.13 (8.41)	43.69 (7.99)	0.402
SICI	16.50 (19.05)	24.73 (31.95)	0.132
ICF	97.46 (19.47)	97.53 (26.61)	0.988
CSP	107.91 (26.34)	109.61 (34.06)	0.788

TS, test stimulus; RMT, resting motor threshold (% of max. stimulator output); SICI, short intracortical inhibition (100% – conditioned MEP/unconditioned MEP × 100%); ICF, intracortical facilitation (conditioned MEP/unconditioned MEP); CSP, cortical silent period (at 110% of RMT, in ms), baseline values of the two sessions were averaged for the analysis of baseline differences between the two genotypes.
[a]t-test for independent samples.

Table 3. Means (SD) of the genotype dependent TMS measures

	BL_PL		PL		BL_CBZ		CBZ	
Variable	AA	GG	AA	GG	AA	GG	AA	GG
TS	57.25 (8.96)	55.07 (7.67)	57.36 (1.27)	55.70 (1.27)	57.67 (8.27)	55.49 (8.17)	59.52 (1.40)	57.90 (1.373)
RMT	44.79 (9.04)	43.84 (8.36)	45.39 (1.26)	43.45 (1.186)	45.41 (8.56)	43.53 (8.92)	47.94 (1.36)	45.53 (1.26)
SICI	15.22 (21.09)	26.66 (39.23)	23.25 (3.573)	31.22 (5.34)	18.01 (27.83)	22.80 (48.41)	14.59 (4.56)	23.05 (4.57)
ICF	97.40 (22.14)	99.69 (35.09)	92.11 (3.26)	95.30 (5.216)	97.61 (30.72)	95.38 (51.45)	94.28 (4.63)	92.21 (4.44)
CSP	107.65 (32.33)	117.70 (42.35)	112.56 (5.279)	109.40 (6.11)	106.88 (27.49)	101.45 (36.75)	113.76 (4.71)	113.87 (6.14)

TS, test stimulus; RMT, resting motor threshold (% of max. stimulator output); SICI, short intracortical inhibition (100% – conditioned MEP/unconditioned MEP × 100%); ICF, intracortical facilitation (conditioned MEP/unconditioned MEP × 100%); CSP, cortical silent period (at 110% of RMT, in ms); BL, baseline; PL, placebo; CBZ, carbamazepine; BL_PL, baseline before placebo; BL_CBZ, baseline before CBZ.

Table 4. Overall effect of CBZ measured in differential reaction to CBZ (compared to placebo and the corresponding measures at baseline)

Variable	Difference in reaction	p[a]
TS	1.9560	**<0.001**
RMT	2.1978	**0.002**
SICI	0.0793	0.526
ICF	−0.2000	0.299
CSP	10.3986	**0.023**

TS, test stimulus; RMT, resting motor threshold (% of max. stimulator output); SICI, short intracortical inhibition (100% − conditioned MEP/unconditioned MEP × 100%); ICF, intracortical facilitation; CSP, cortical silent period (at 110% of RMT, in ms), bold, $p < 0.05$.
[a]Paired samples *t*-test.

and CSP showed a differential reaction to CBZ (TS: $t(90) = 3.80$, $p < 0.001$; CSP: $t(80) = 2.311$, $p = 0.023$, Table 4). There was no significant overall effect of CBZ on the TMS parameters SICI and ICF ($p > 0.05$, Table 4). As expected, subjects experienced more side effects after intake of CBZ than after intake of placebo (3.70 ± 3.88 vs. 2.01 ± 2.67; $t(87) = -4.36$, $p < 0.001$). There was no correlation of side effects with gender, CBZ level, or genotype (all $p > 0.1$). The most common side effects of CBZ were described by the volunteers as slight effects, such as sleepiness, coordination difficulties, and vertigo.

Genotype-dependent effects of CBZ

A MANCOVA with the differences in change in RMT and CSP from baseline to post medication (CBZ-PL) as dependent variables, and the covariates gender, CBZ level, and TS at CBZ measure with the two-stage-factor genotype (AA vs. GG) revealed a significant main group effect ($p = 0.029$; Table 5). Univariate analysis showed that volunteers with genotype GG had a higher increase in CSP duration compared with genotype AA after intake of CBZ as compared to placebo (21.53 ± 6.31 msec vs. 0.56 ± 5.93 msec, $p = 0.013$, Fig. 1). There were no group differences in changes of RMT after intake of CBZ as compared to placebo ($p = 0.813$). Furthermore, SICI, ICF, and TS did not vary significantly by genotype ($p > 0.05$).

Discussion

This study demonstrates that changes in cortical excitability after intake of the sodium-channel blocker CBZ differ in subjects with different genotypes of the common *SCN1A* splice-site polymorphism rs3812718. In particular, CBZ induced a significant CSP prolongation in subjects with genotype GG, when compared to AA. This is the first demonstration of a pharmacogenetic functional link between this *SCN1A* SNP genotype and CBZ response, which may underlie the reported correlation of SNP genotype and antiepileptic drug efficacy in epilepsy patients.[8,12] The expected genotype-independent increase in the TMS parameters RMT and CSP reflects effects on voltage-gated sodium channel function in general and is confirmatory of previous reports.[16,26] No significant baseline differences in cortical excitability were observed.

Because the CSP depends on the stimulus intensity used as well as the intensity of voluntary muscle contraction of the probands, a reproduction of the results by further studies would be desirable.

Genotype-dependant effects of CBZ

The *SCN1A* polymorphism rs3812718 has been found to be associated with changes in electrophysiologic and pharmacologic properties of the sodium channel $Na_v1.1$.[3,8,10] By disrupting the splice donor consensus sequence directly following the neonatal splice variant of exon 5 (5N), the A allele leads to significantly decreased expression of the neonatal exon 5N relative to the adult exon 5A.[8,9]

Heterologously expressed $Na_v1.1$-5N channels have been shown to display enhanced tonic and use-dependent blocks by phenytoin and lamotrigine, when compared to $Na_v1.1$-5A channels.[13] These data provide a pathophysiologic basis at the molecular level for the decreased effectiveness and higher required dose of sodium channel blocking anticonvulsant drugs in epilepsy patients with genotype AA, which has been reported in some,[8,12] but not all, clinical studies.[14,15] The present study extends the mechanism of this association to the network level. Our data support an altered effectiveness of sodium-channel blockers depending on the rs3812718 genotype.[8,12] Using in vivo measurements in human subjects, we show that this differential effect may involve the GABAergic system, as the later components of the CSP are mainly modified by changes in GABAergic inhibition of pyramidal cells through interneurons.[16,18,27–29]

Although the most robust effect of CBZ in this and previous studies was on the RMT, which reflects changes in

Table 5. Effects of MANCOVA with the group factor "genotype AA versus GG" of RMT and CSP with gender, CBZ-level and TS under CBZ as covariates

Source of variation	Dependent variable	F	d.f.1	d.f.2	p	η^2
Main effect: "AA versus GG"	Multivariate analysis	3.73	2	75	**0.029**	0.090
	RMT	0.06	1	76	0.813	0.001
	CSP	6.55	1	76	**0.013**	0.079

d.f., degrees of freedom; η^2, effect size; TS, test stimulus; RMT, resting motor threshold; CSP, cortical silent period; CBZ, carbamazepine, bold, $p < 0.05$.

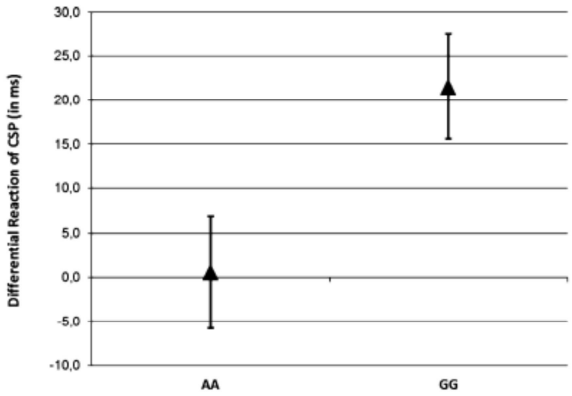

Figure 1.
Differential reaction of the cortical silent period to carbamazepine depending on genotype. Mean and SD (error bars) for the significant ($p = 0.013$) difference in reaction to the two treatments (CBZ-Placebo); the group with genotype GG showed an increased CSP after CBZ versus placebo, whereas in the group with genotype AA, the CSP remained unchanged compared to the placebo treatment. CSP, cortical silent period (at 110% of RMT, in msec); GG, genotype GG; AA, genotype AA; CBZ, carbamazepine.

voltage-gated sodium channels in general,[16,18,28,30] we unexpectedly did not find a differential effect of the rs3812718 genotype on CBZ-induced RMT change. However, in mouse models a mutation (R1648H) or knockout of *SCN1A* alters electrophysiologic properties and reduces sodium channel function in GABAergic interneurons, but not excitatory pyramidal cells.[1,31,32] This is likely due to the differential expression of the sodium channel $Na_v1.1$[1] and suggests that mutations in the *SCN1A* gene in general, as well as the polymorphism rs3812718 studied here, mainly affect inhibitory interneurons expressing $Na_v1.1$., which is consistent with the genotype-dependent changes in CSP found in this study. In contrast, the excitability of pyramidal cells determining the RMT remained unaffected by this variation in *SCN1A*.

SCN1A and pharmacoresistance

Pharmacoresistance is an important factor in the treatment of epilepsy, affecting about 30% of patients[33] and resulting in increased suffering, mortality, and treatment costs.[34] Two main hypotheses explaining pharmacoresistance have been proposed: (1) the transporter hypothesis, implying a lower brain concentration of AED due to altered transport across the blood–brain barrier, and (2) the substrate hypothesis, suggesting decreased responsiveness of the drug target, for example, due to structural alteration.[34] Our results provide further evidence for an association of the rs3812718 polymorphism with the pharmacoresponse and lend support for the substrate hypothesis.

Baseline differences

In addition to the altered pharmacologic properties of $NA_v1.1$ related to the *SCN1A* polymorphism rs3812718, there are also reports of electrophysiologic changes in drug-naive physiologic conditions.[10] Such genotype-dependent electrophysiologic differences would suggest that rs3812718 may confer susceptibility to epilepsy. Accordingly, previous studies reported an association between the A allele of rs3812718 and febrile seizures,[3,4] and a genome-wide association study revealed an association between chromosomal regions encompassing the *SCN1A* gene and GGE.[7] These findings support electrophysiologic changes associated with *SCN1A* polymorphisms and their possible role in the pathogenesis of genetically complex forms of generalized epilepsies.

However, other studies could not replicate an association between rs3812718 and febrile seizures.[5,6] Furthermore, a recent patch-clamp investigation examining the electrophysiologic properties of $Na_v1.1$ did not show significant electrophysiologic differences in drug-naive conditions including voltage dependence of activation, steady-state inactivation, and recovery from inactivation between the two genetic variants of $Na_v1.1$.[13] Changes in electrophysiologic properties associated with rs3812718, therefore, remain controversial.

We investigated possible in vivo baseline differences in cortical excitability in healthy subjects with genotypes AA and GG using TMS. None of the TMS parameters applied revealed significant differences in cortical excitability before application of CBZ or placebo. Our study does not lend support to the idea of baseline differences in cortical excitability accompanying the different genotypes of rs3812718, at least not in a range or manner that can be measured by TMS.

Conclusion and Open Questions

Our results provide evidence of increased cortical inhibition after intake of CBZ in subjects with genotype GG as compared to AA of the *SCN1A* polymorphism rs3812718 while showing no baseline differences in cortical excitability. The results imply that the higher doses of CBZ in patients with genotype AA that were described in earlier studies are not due to an increased baseline excitability, but rather a differential effect of CBZ that is dependent on the *SCN1A* genotype.

These data provide insight into the physiology underlying pharmacoresponse, supporting the substrate hypothesis and relating the altered effectiveness of CBZ to changes in the excitability of GABAergic interneurons. Furthermore, this study shows that TMS is a useful method to gain insight into the influence of genetic variants on cortical excitability and pharmacoresponse.

The present study only included subjects with the homozygous genotypes AA and GG and showed that these are

related to changes in pharmacoresponse that can be measured by TMS. Considering these results, future studies should also include subjects with the heterozygous genotype AG to further characterize the effect of this polymorphism.

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Disclosure

The authors declare the following: KM has received a travel grant and speaker honoraria from GlaxoSmithKline, which are unrelated to this study. CD has received speaker honoraria from the Epilepsy Center Hessen and a travel grant from Eisai, unrelated to this study. SS has nothing to disclose. She is a member of the Epilepsy Research United Kingdom Science Advisory Committee and the UCL council and has received a Royal Society Fellowship unrelated to this study. PSR has received travel grant from UCB. KMK has received a scholarship from the University of Melbourne and speaker honoraria from the Epilepsy Center Hessen. AHa has received honoraria for data analysis, manuscript preparation for an observational study from UCB, and for a survey study from Sanofi-Aventis. She was supported by a research fellowship from the German Research Foundation (DFG. Ha 6363/1-1).

In the past 2 years WHO has received speaker honoraria from Boehringer Ingelheim, Desitin, GlaxoSmithKline, Orion, Novartis, UCB Pharma/Schwarz Neuroscience, and Teva. He also has received honoraria as scientific advisory (consultant) from Desitin, Merck Sharp and Dome, Merck Serono, Novartis, Orion, UCB Pharma/Schwarz Neuroscience, and Teva.

HMH has received honoraria as advisory board member from Desitin, GSK, Eisai, UCB, and Pfizer, and research grants from Desitin, Janssen Cilag, and UCB. He has received speaker honoraria from several companies and institutions, such as University of Munich, University of Kiel, University of Saarbrücken, Desitin, Eisai, GSK, Pfizer, Novartis, Nihon Kohden, AdTech, and UCB. He has received payment for manuscript preparation from Pfizer and development of educational presentations from UCB. SK She has received speaker honoraria from UCB, Desitin, und Eisai, which are unrelated to this study.

Within the past 2 years, FR has received honoraria as scientific advisor from GSK, Eisai, UCB, and Pfizer. He has received speaker honoraria from UCB, GSK, Eisai, Desitin, and Medtronic, and educational grants from Nihon-Kohden, UCB, Medtronics, Cyberonics, and Cerbomed. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Side effect score.

Test-Retest Reliability of Single and Paired Pulse Transcranial Magnetic Stimulation Parameters in Healthy Subjects

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Key words: gender, automated cortical silent period, cortical excitability, retest interval

Number of words (max. 4000): 3652

Number of words (Abstract, max. 250): 250

Short title: Test-Retest Reliability in TMS

Abstract

Background: Transcranial magnetic stimulation (TMS) is widely used to assess cortical excitability. Studies on test-retest reliability mainly included small samples and lacked definition of potential influencing factors such as daytime of measurement, other investigator effects, and women were frequently not included.

Objective: To determine test-retest reliability of different TMS parameters and several confounding factors (investigator, retest-interval and gender) while controlling potential confounders (time of day and period of menstrual cycle in women) in a large sample of healthy subjects and to compare visually guided and automated methods for cortical silent period (CSP) duration.

Methods: Four investigators applied TMS in 93 healthy volunteers twice (57 male, 61%) with a retest interval of 34.0 ± 25.6 (SD) days, mainly in the morning hours. Women were assessed in their follicular phase. Test stimulus (TS), resting motor threshold (RMT), short latency intracortical inhibition (SICI), short latency intracortical facilitation (SICF) and CSP, both visually and automated, were analyzed.

Results: Good test-retest reliabilities were observed for TS (r=.880) and RMT (r=.826). Visual (r=.466) and automated (r=.486) analyzed CSP durations showed comparable moderate reliability. SICI (r=.383) and SICF (r=-.159) had lower correlations. Reliabilities were different across investigators for CSP and low when TMS was applied by different investigators across sessions.

Conclusions: In a large sample of healthy volunteers we found moderate to strong test-retest reliabilities in all excitatory and in all but one (SICF) inhibitory TMS-parameter with investigator as main confounding factor. Automated analysis of the CSP did not prove to be more reliable than visual determination.

Introduction

Cortical excitability is frequently assessed using transcranial magnetic stimulation (TMS) for clinical and research purposes (Rossini&Rossi, 2007). However, to allow the detection of relevant and clinically meaningful changes in TMS parameters and thus cortical excitability, sufficient test-retest reliability is required. Confounding factors that may systematically influence TMS results and hence reduce reliability should be recognized and considered when developing TMS-based research protocols.

Several studies have looked at variability in TMS before. Those studies have found overall good reliabilities of TMS parameters in single and paired-pulse settings in healthy volunteers (Carroll et al., 2001; Maeda et al., 2002; Cacchio et al., 2009; Farzan et al., 2010) as well as in stroke patients (Wheaton et al., 2009; Cacchio et al., 2011) and amputees (Hetu et al., 2011). Resting motor threshold (RMT) was commonly found to be very reliable (Malcolm et al., 2006; Plowman-Prine et al., 2008; Cacchio et al., 2009; McGregor et al., 2012) with few results at variance (De Gennaro et al., 2003). The RMT is defined as excitability of cortico-cortical axons and their connection to pyramidal cells (Ziemann, 2004). Mainly voltage-gated sodium channels mediate this REF.

Studies regarding reliability of the cortical silent period (CSP) are sparse (Sale et al., 2007; Cacchio et al., 2009; Farzan et al., 2010). CSP, here measured as contralateral interruption of tonic contractions of the hand muscle, represents both spinal inhibitory processes during the first 50ms and cortical mechanisms as motor cortex inhibition later than 100ms (Wolters et al., 2008). The latter is thought to reflect inhibition of pyramidal cells $GABA_B$ receptors through interneurons (Ziemann et al., 1996; Werhahn et al., 1999; Ziemann, 2004).

and studies on reliability of short latency intracortical inhibition (SICI) and facilitation (SICF) have been inconclusive (Boroojerdi et al., 2000; Maeda et al., 2002; Wassermann, 2002; Ngomo et al., 2012).

Both SICI and ICF result when using a double-pulse design. A subthreshold conditioning stimulus (CS) is followed by the suprathreshold individual test stimulus (TS) with varying

interstimulus intervals. ICF can be elicited with ISI 6-25 ms while SICI is tested at 1-6ms (Reis et al., 2008).

The variability in TMS results might stem from inherent differences between participants (18, 22-24). In women, TMS has revealed that menstrual cycle phase affects cortical excitability and inhibition (Smith et al., 1999; Cahn et al., 2003; Hattemer et al., 2007). However, only a few TMS studies on retest-reliability included women (Maeda et al., 2002; De Gennaro et al., 2003; Christie et al., 2007; Siniatchkin et al., 2011) and only one specified the phase of the menstrual cycle during which TMS was performed (Siniatchkin et al., 2011). DeGennaro (De Gennaro et al., 2003) argued that their low reliabilities were not result of including women as the male participant also showed low reliabilities. Additionally, investigators' skill and expertise in neurophysiological research were suggested to be relevant (Cacchio et al., 2009), but investigator effects have not yet been formally studied.

Lately, the proposed method of choice for determination of the CSP duration has been automated computer analysis rather than visual analysis to reduce variability (Garvey et al., 2001; Daskalakis et al., 2003; King et al., 2006). Yet, to the best of our knowledge, there is no study comparing both methods regarding test-retest reliabilities hence no superiority claim can be made.

Additionally, studies on retest reliability have discussed several factors potentially influencing measures of cortical excitability, including age, daytime and handedness (Maeda et al., 2002; Wassermann, 2002; Christie et al., 2007; Pfutze et al., 2007; Sale et al., 2007; McGregor et al., 2012; Reid&Serrien, 2012) and period of menstrual cycle in women (Hattemer et al., 2006; Hattemer et al., 2007). Controversy exists for age, as it correlated negatively with reliability in one (McGregor et al., 2012) but not in other studies (Wassermann, 2002; Christie et al., 2007). One study in patients with juvenile myoclonic epilepsy did not find differences between morning and afternoon measures (Pfutze et al., 2007) while another found measurements during the afternoon more reliable (Sale et al., 2007). Data on handedness also remained inconclusive with no detected influences of the hemisphere tested on reliability

in one paper (Maeda et al., 2002) while a recent study (Reid&Serrien, 2012) demonstrated differences in inhibition due to handedness.

We therefore chose to control for age by including only young adults, daytime by measuring only during the morning, handedness by only including right-handed individuals and menstrual cycle by measuring during the follicular phase to yield precise data on our main aim on retest reliability.

We hypothesized, due to the above named data, that retest reliability would be strong for RMT and CSP, while SICI and ICF would be moderate.

Methods

Subjects

Exclusion criteria involved a history of neurological and/or psychiatric disease and use of central nervous system active drugs. Only right-handed subjects with a score ≥ 80 on the Edinburgh handedness inventory (Oldfield, 1971) were included to have a homogenous sample for stimulation site, namely only the suspected dominant left hemisphere.

Participants consented to refrain from caffeine intake or smoking for 12 hours prior to assessments. All female participants underwent both TMS sessions during the follicular phase as determined by individual calendars.

The study conformed to the declaration of Helsinki and was approved by the local ethics committee of the Philipps-University Marburg, Germany. Written informed consent was obtained from all participants.

Ninety-six participants were included in the study. After excluding three participants (one due to technical problems, two did not finish the study) results from 93 volunteers (female n=36, 38.7%; male n=57, 61.3%, age: 23.74 ± 3.38 years, range: 19-36 years) were analyzed.

Investigators

Four investigators applied TMS in this study. All investigators received training from two experienced supervisors. For training purposes, all investigators applied TMS and analyzed data of several volunteers before they acquired data for the present study. Throughout the whole study, one experienced investigator was always available for support. The numbers of participants per investigator are displayed in table 1.

Table 1 approx. here

Sessions

Each participant completed two sessions (T1, T2). These were conducted at minimum of 14 days apart. TMS was repeated on average 34.0 ± 25.6 days after the first session (range 14 – 173 days). The two sessions represent baseline measurements for an experimental study on carbamazepine induced acute changes of cortical excitability (Menzler et al., 2014). All participants were assessed between 7am and 2pm. On average, the second session started 30 minutes earlier than the first (10:19h ± 1.26h vs. 9:49h ± 1:21 h, p=.005).

Transcranial magnetic stimulation

Subjects were comfortably seated in an armchair with the head fixed in a custom plastic foam headrest. TMS was delivered through a focal figure-of-eight shaped magnetic coil (70mm external loop diameter) connected to two Magstim 200 magnetic stimulators via a BiStim-module (all Magstim, Whitland, Dyfed, UK). The coil was placed flat on the head over the left motor cortex, at an approximate angle of 45° to the sagittal plane, inducing a current in the brain roughly perpendicular to the central sulcus, flowing from posterior to anterior, as this has been reported to be the most effective way to activate the corticospinal system transsynaptically (Brasil-Neto et al., 1992). Motor evoked potentials were recorded using surface EMG Ag/AgCl electrodes placed over the right abductor digiti minimi muscle (ADM) in a belly-tendon montage. The raw signal was amplified, filtered (20Hz-10kHz) and recorded

with a PC using a commercially available data-collection and averaging program (Magnetix®, Center of Sensorimotor Research, Munich, Germany) for offline analysis.The optimal coil placement was determined by recording motor evoked potentials (MEP) while varying the coil position. The coil position leading to the highest peak-to-peak amplitude of the MEP (´hot spot´) was marked with a semi-permanent pen directly on the scalp to ensure accurate coil positioning throughout the testing.

All sessions followed a fixed sequence of TMS measurements: First, TS and RMT, then the paired-pulse parameters, SICI and SICF, were obtained in random order. In all paired pulse TMS procedures, the interval between trials was randomly changed between 4 and 6 s, in single pulse procedures the inter-trial interval was 5 s. The protocol concluded with determination of the CSP.

TMS-parameters

TMS parameters were specified as follows:

1. The resting motor threshold (RMT) was defined as the lowest stimulator output intensity that induced MEP peak-to-peak amplitude greater than 50 μV in at least five of ten consecutive trials. Complete muscle relaxation was monitored via audiovisual feedback. A step-by-step intensity resolution of the maximal stimulator output was used for determination of the individual RMT using the maximum likelihood threshold hunting (MLTH) procedure for TMS (Friedemann Awiszus, Magdeburg (Awiszus, 2003)).
2. Short intracortical inhibition (SICI) and short intracortical facilitation (SICF) were obtained with paired-pulse TMS. A conditioning and a test stimulus were applied with different fixed interstimulus intervals (ISI). The conditioning stimulus was set to an intensity of 75% of the RMT as this does not produce changes of excitability in the spinal cord (Kujirai et al., 1993; Di Lazzaro et al., 1998). The intensity of the following suprathreshold test stimulus was adjusted to produce MEPs of approximately 1.5 mV

peak-to-peak amplitude if delivered without preceding conditioning stimulus (test stimulus, TS). SICI was obtained at short ISIs of 3ms, leading to a decreased MEP as compared to a MEP induced by a non-conditioned test stimulus. SICF was obtained using ISIs of 10 ms, leading to an increased MEP (Brasil-Neto et al., 1992; Kujirai et al., 1993; Nakamura et al., 1997; Werhahn et al., 1999; Reis et al., 2004). Fifteen trials of single non-conditioned test stimuli and fifteen paired stimuli of each ISI, generated in random order by the computer program, were recorded. The average of the 15 trials was used to define the amplitude of the peak-to-peak MEP for each condition. The conditioned response was defined as the mean amplitude of the conditioned responses belonging to each ISI, expressed as percentage of the mean amplitude of the unconditioned test response. For better comparability, this percentage was subtracted from 100% for SICI [SICI: 100% - (conditioned response/unconditioned response x100%); SICF: conditioned response/unconditioned response x100%; (Wahl&Ziemann, 2007)]

3. The cortical stimulation-induced silent period (CSP) was measured during 20 trials at a stimulus intensity of 110% of the RMT. Participants were instructed to hold a voluntary muscle contraction of approximately 30% of their maximal force, controlled by audio-visual feedback. CSP duration was determined offline in two ways. For visually guided analysis, the CSP duration was defined as the time from TMS stimulus artefact to the first reoccurrence of voluntary EMG activity exceeding 25% of muscle activity prior to the stimulus. This was always determined by the same investigator in order to minimize variability. Duration was determined offline in the Magnetix® program.

   Additionally, automated analysis of the CSP was carried out with custom software (CSPDuration©, C. Bauer, Schopp, Germany) based on the method introduced by Garvey et al (Garvey et al., 2001). Briefly, the twenty individual curves are single-trial full-wave rectified and averaged. Via an algorithm, the duration of the CSP is determined by mean pre-stimulus EMG, mean consecutive difference and a constant

of 2.66. CSPDuration© default for averaging was 51 points. The program ignores calculated durations of the CSP of the original algorithm that are shorter than 30ms or have a starting point more than 250ms post MEP. The beginning of the CSP was set after the MEP.

All participants therefore had two separate analysis of the CSP- visually and automated.

One single-value per person per session was calculated by averaging separately for RMT, SICI, SICF, visual CSP, and automated CSP.

Statistical analysis

Analysis was computed with PASW® Statistics 20® (SPSS, IBM Company, Chicago, Illinois). Data are displayed as Mean ± SD.

Comparison of daytime of measurement during first and second session was calculated by paired t-tests.

Multivariate analysis of variance (MANOVA) was applied with the 4-staged between subject factor investigator (A, B, C, D) and the within-subject factor session (T1, T2) and TS, RMT, SICI, SICF, visual CSP, automated CSP as dependent variables. Furthermore a MANOVA was conducted with the same dependent variables and within-subject factor but gender as the between-subject factor.
If group variances were non-homogenous (according to Mauchly´s test of sphericity), degrees of freedom were adjusted (Greenhouse-Geisser).
When appropriate, either univariate analysis of variance or post-hoc tests (Bonferroni adjusted) are reported.

Normalization of the data was conducted for a second analysis as follows: per session and investigator the mean of RMT, visual CSP and automated CSP were computed and the individual scores of participants divided and given in percentages, e.g. RMT at T1= RMT_T1 / (mean of RMT_T1 per investigator) x 100.

Reliability for all TMS parameters was calculated using Pearson´s product moment correlation coefficient (r) as suggested by Rousson et al (Rousson et al., 2002). For the reliability-analysis outliers were not considered in the calculation. They were first selected by eye in the scatterplot and confirmed statistically (> mean ± 2 SD). This resulted in excluding one participant for RMT analysis (n=92) and two participants for SICI (n=91) analysis.

The level of significance was set to p=.05 (two-tailed).

Results

Multivariate analysis of variance (MANOVA) with investigator as 4-staged between-subject factor and session as within-subject factor indicated a significant main investigator effect ($p<.001$, table 2), while no effects of session or interaction were found ($p>.1$). In univariate analysis the investigator effect held only for the RMT ($F(3, 89)=5.404$, $p=.002$). Post-hoc tests revealed that investigator D obtained higher RMTs than investigators A ($p=.001$) and B ($p=.003$) and by trend also C ($p=.059$). No systematic differences between sessions were observed.

Table 2 approx. here

Normalization of the data

When normalizing the data per investigator to determine the individual deviation from the norm of the investigator, the RMT, visual CSP and automated CSP were included in a repeated measures MANOVA. The group effect of investigator remained significant (F (9,

211.886) =4,081, p=.000) while the effect of session and the interaction effect continued insignificant (p>.1). In univariate analysis, the investigator effect held for the RMT (p=.002).

Overall test-retest reliability

The reliability analysis disclosed significant correlations between session 1 and 2 for all TMS-parameters (table 3) except SICF (r=-.159, p=.127). Good correlations for TS (r=.880) and RMT (r=.826, figure 1) were reached.

Figure 1 approx. here

Visual vs. automated CSP

Considering the whole sample, both visual and automated CSP were moderate in reliability (respectively r=.466, r=.486, figure 2).

Figure 3 approx. here

Confounding factors

Re-test interval

Regarding reliability of short (<28 days, Median Split) vs. long retest intervals (≥ 28 days) we found the same parameters to be reliable as in the overall analysis (table 3). The two techniques of CSP-determination revealed that automated analysis prevailed on overall retest reliability and short delay (<28 days) repeated measurements.

Investigator

The four investigators measured different numbers of participants (see table 3). All achieved good reliabilities for TS and, for three investigators, RMT. In three investigators moderate reliabilities were reached for visual and automated CSP while one investigator presented low reliabilities (r=.205, see table 3). SICF presented the lowest results while SICI was slightly better but variable across different investigators.

Table 3 approx. here

Change of investigator across sessions which occurred in five participants led to diminished reliabilities in almost all parameters (table 3). RMT was the most stable TMS-parameter, and both automated and visual CSP were poorly reliable when the repeated TMS was conducted by a different investigator.

Gender

A repeated measures MANOVA with gender as between-subject factor, session as within-subject factor (T1, T2) and six dependent variables (TS, RMT, SICI, SICF, visual CSP, automated CSP) revealed an effect of gender ($F(6,84)=3,39$, $p=.005$). However, a univariate analysis showed all $p>.1$. Reliabilities on TS (women: $r=.867$, men: $r=.894$, both $p<.001$) and RMT (women: $r=.819$, men: $r=.837$, both $p<.001$) were comparably high. Moderate reliabilities were reached on SICI (women: $r=. 333$, $p=.05$; men: $r=.410$, $p=.002$), visual CSP (women: $r=.477$, $p=.004$; men: $r=.408$, $p=.002$) and automated CSP (women: $r=.538$, men: $r=.422$; both $p=.001$).

Discussion

This study evaluated test-retest reliability during single and paired pulse TMS in a large sample of healthy right-handed male and female participants and determined influences of short versus long retest interval, change of investigator and visual versus automated analysis of the CSP. We controlled our study for potential influences of gender, daytime of TMS, age, handedness and period of the menstrual cycle in women. Before calculating test-retest reliabilities we confirmed that both TMS sessions were held under the same conditions as we did not find any systematic differences between the TMS parameters collected during the first and second session.

We found overall strong correlations between the two sessions for TS and RMT. Both visual and automated analysis of CSP as adapted from Garvey et al (Garvey et al., 2001), provided evidence for moderate retest reliability of this parameter with no substantial difference between the two methods. The short intracortical inhibition (SICI, at ISI 3 ms) yielded moderate correlations while the short intracortical facilitation (SICF, at ISI 10 ms) was not reliable.

In line with other research the RMT was a very reliable parameter (Cacchio et al., 2009) which supported its frequent use as key variable in TMS research. This was the case for short and long retest intervals and also when the investigator changed. The higher RMT measured consistently by one investigator suggests to use individual differences (RMT post - RMT pre intervention) for statistical analysis rather than absolute scores when several investigators contribute data to an interventional study.

Most of the studies on test-retest reliability of TMS either do not provide any data on CSP (Boroojerdi et al., 2000; Carroll et al., 2001; Maeda et al., 2002; Cahn et al., 2003; De Gennaro et al., 2003; Wolf et al., 2004; Malcolm et al., 2006; Plowman-Prine et al., 2008; Siniatchkin et al., 2011; McGregor et al., 2012) or do not specify whether offline determination of duration of CSP was conducted visually or with an automated algorithm (Sale et al., 2007; Cacchio et al., 2009; Farzan et al., 2010). While lately the proposed

method of choice is automated CSP analysis, to our knowledge, no retest reliabilities were published. By providing data on two methods of CSP analysis we showed that both displayed moderate retest reliability. While overall correlation for the automated CSP was slightly stronger the difference was not relevant. Interestingly, the CSP seemed to be fairly independent of the interval between measurements but was more dependent on the investigator than the RMT. Additionally, the investigator was more relevant to the retest reliability of the CSP than the mode of data analysis (visual vs. automated). In conclusion. our data suggest that repeated measures should whenever possible be obtained by the same investigator rather than emphasizing the method of analysis.

Our finding of low reliabilities of the SICF are in concordance with one earlier study, confirming that shorter ISIs are more reliable than longer ISIs (Maeda et al., 2002) while another study found SICF to be less variable (Orth et al., 2003). The reasons for this disconcordance remain to be determined. Maybe testing other ISIs could have strengthened the results.

Concerning the influence of the investigator, our data illustrates that those with less experience can reach good retest reliability for TS and RMT, after receiving training by more experienced investigators, despite the attribution of strong correlations to profound experience reported in another study (Cacchio et al., 2009). For measures of CSP, it remained more variable across investigators. It was not influenced by cumulative experience in measurements, as the investigator with most sessions displayed lowest correlations. Several studies have underlined the confounding potential of hormone changes during the menstrual cycle in menstruating women (Smith et al., 1999; Wassermann, 2002; Cahn et al., 2003), although this ovarian hormone dependent change was not present for the MEP threshold. The present study, to our knowledge, provides data of the largest cohort of women, all measured during their follicular phase to minimize effects of ovarian hormones. Given this standardization reliabilities were equally good in women as in men, suggesting that, at least when measuring in the same phase of the hormonal cycle, reliabilities are high.

More studies are needed to explore the retest reliabilities in elder vs. younger, right- vs. left-handed individuals and during different phases of the menstrual cycle as these variables were held stable during this study to minimize their potential impact on retest reliabilities.

In conclusion, this study corroborates strong retest reliabilities in single and paired-pulse TMS, especially for TS and RMT, and provides insights into the effects of different investigators, gender and retest intervals on test-retest reliability in a large sample of healthy right-handed individuals. The comparison of test-retest reliability of visual vs. automated analysis of CSP duration demonstrated here for the first time that it was more dependent on the investigator than the analysis method used.

These data can be helpful to determine the significance of observed changes in cortical excitability as well as for study design and sample size calculation in future research.

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Conflicts of interest

The authors declare the following conflicts of interest:

AHe has no conflicts of interest to declare.

AHa has received honoraria for data analysis, manuscript preparation for an observational study from UCB and for a survey study from Sanofi-Aventis.

CD, KB and HB have no conflicts of interest to declare.

SB and VM have no conflicts of interest to declare.

Within the last two years FR has received honoraria as scientific advisor from GSK, EISAI, UCB, Pfizer. He has received speaker honoraria from UCB, GSK, EISAI, Desitin, Medtronic and educational grants from Nihon-Kohden, UCB, Medtronics, Cyberonics and Cerbomed. FR has, however, no COI regarding this study.

Legends

Table 1. Investigator and number of measurements per session

Investigator	Session 1 (n)	Session 2 (n)	Total sessions (n)
A	25	22	47
B	46	49	95
C	14	14	28
D	8	8	16

Table 2. Effects of MANOVA with the between-group factor "investigator A vs. B vs. C vs. D" and the within-group factor "session 1 vs. 2" on TS, RMT, SICI, SICF, visual CSP and automated CSP

Source of variation	Dependent variable	F	df1	df2	p	eta^2
main effect: "investigator A vs. B vs. C vs. D"	multivariate analysis	4.07	18	258	**.000**	.223
	TS	.061	3	89	.980	.002
	RMT	5.40	3	89	**.002**	.154
	SICI	1.27	3	89	.290	.041
	SICF	.676	3	89	.569	.022
	Vis_CSP	1.02	3	89	.388	.033
	Aut_CSP	.069	3	89	.976	.002
main effect: "session 1 vs. session 2"	multivariate analysis	1.56	6	84	.170	.100
interaction effect: "session x investigator"	multivariate analysis	1.06	18	258	.397	.070

MANOVA: multivariate analysis of variance, TS: test stimulus, RMT: resting motor threshold, SICI: short latency intracortical inhibition, SICF: short latency intracortical facilitation, Vis_CSP: visual cortical silent period, Aut_CSP: automated cortical silent period.

Table 3. Test-retest reliabilities

	total	Retest interval <28 days (n=50)[1]	Retest interval ≥28 days (n=43)[1]	Same investigator (n=88)[2]	Different investigator (n=5)[2]
TS	.880**	.922**	.825**	.882**	.833
RMT	.826**	.850**	.798**	.824**	.782
SICI	.383**	.230	.532**	.383**	.292
SICF	-.159	-.068	-.258	-.163	-.200
Vis_CSP	.466**	.386*	.572**	.474**	.159
Aut_CSP	.486**	.426*	.565**	.502*	.198
			Investigator		
		A (n=25)	B (n=46)	C (n=14)	D (n=8)
TS		.955**	.844**	.845**	.930*
RMT		.854**	.832**	.578*	.849*
SICI		.717**	.306*	.048	-.268
SICF		.036	-.193	-.018	-.244
Vis_CSP		.600*	.313*	.760*	.605
Aut_CSP		.711**	.205	.798*	.515

*p<.05, **p<.001; same investigator: same investigator during session 1 and session 2; [1]n=49 for short RMT and for short SICI, n=42 for long SICI; [2]n=87 for same RMT, n=86 for same SICI; TS: test stimulus, RMT: resting motor threshold, SICI: short intracortical inhibition; SICF: short intracortical facilitation; Vis_CSP: visually analyzed cortical silent period; Aut_CSP: automatically analyzed cortical silent period

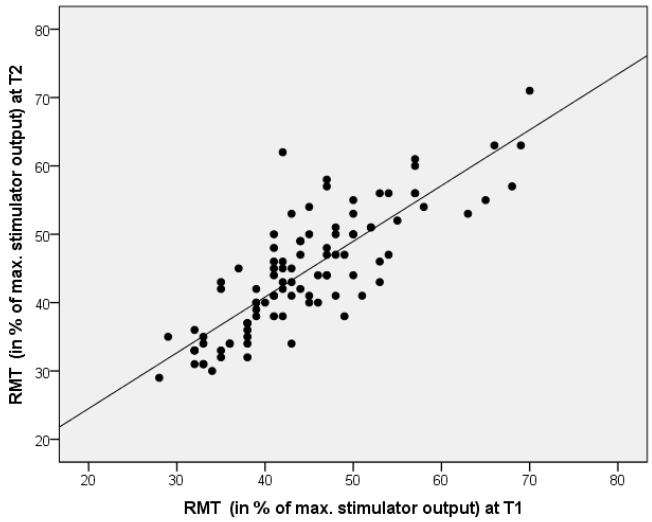

Figure 1. Significant correlation (p<.001) between the two sessions for resting motor threshold (RMT, r=.880) The RMT is displayed in % of maximum stimulator output. T1=first session, T2= second session

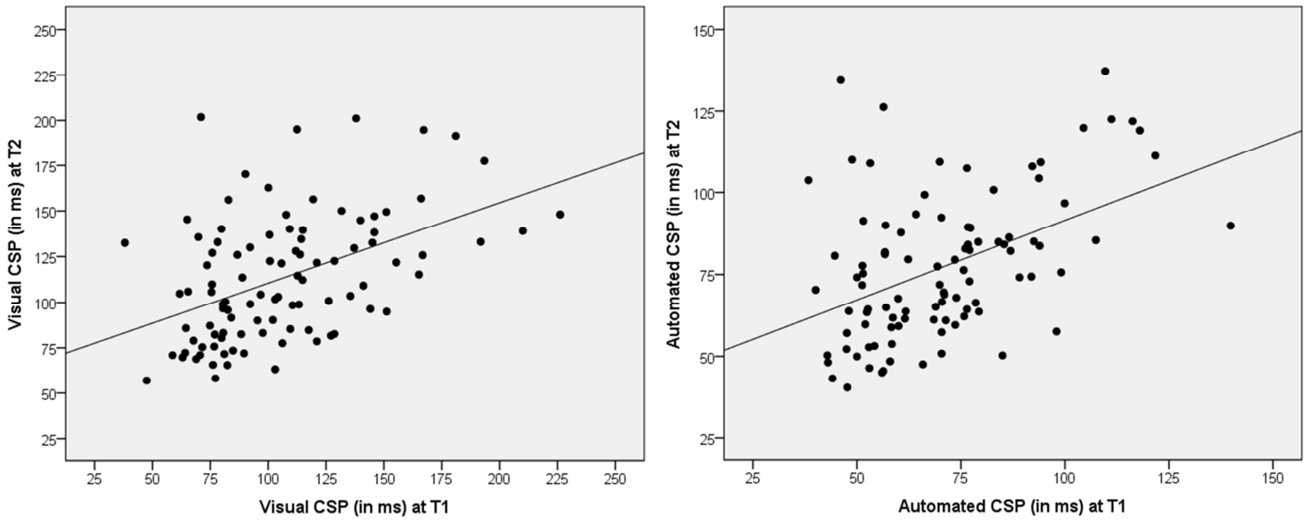

Figure 2. Significant correlation (both p<.001) between the two sessions for visual (left) and automated cortical silent period (CSP, respectively r=.466 and r=.486). Both are duration of CSP in ms. T1=first session, T2= second session